European Medicines Agency
*Pre-authorisation Evaluation of Medicines for Human Use*

London, 23 July 2009
Doc. Ref. EMEA/CHMP/BWP/472717/2009

# REPORT

# EXPERT WORKSHOP ON CJD RISK AND URINE-DERIVED MEDICINAL PRODUCTS

## EMEA, London - 12-13 July 2007

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 85 45
E-mail: mail@emea.europa.eu http://www.emea.europa.eu

## TABLE OF CONTENTS

**GLOSSARY**

| | |
|---|---|
| BSE | Bovine Spongiform Encephalopathy |
| CHMP | Committee for Medicinal Products for Human Use |
| CJD | Creutzfeldt-Jakob Disease |
| CNS | Central Nervous System |
| CWD | Chronic Wasting Disease |
| EAE | Experimental Autoimmune Encephalomyelitis |
| GBR | Geographical BSE Risk |
| GSS | Gerstmann-Sträussler-Scheinker disease |
| hCG | Human Chorionic Gonadotropin |
| IC | Intracerebral |
| iCJD | Iatrogenic CJD |
| ID | Infectious Dose |
| IP | Intraperitoneal |
| IV | Intravenous |
| NCJDSU | UK National CJD Surveillance Unit |
| PCR | Polymerase Chain Reaction |
| PI | Isoelectric Point |
| PK | Proteinase K |
| PMCA | Protein Misfolding Cyclic Amplification |
| PrP | Prion Protein |
| $PrP^{C}$ | Cellular Prion Protein |
| $PrP^{Sc}$ | Pathogenic Misfolded Isoform |
| $PrP^{TSE}$, $PrP^{RES}$, $PrP^{d}$ | Abnormal disease associated prion protein |
| SC | Subcutaneous |
| SCEPA | Scrapie Cell End Point Assay |
| sCJD | Sporadic Creutzfeldt-Jakob Disease |
| TME | Transmissible Mink Encephalopathy |
| TSE | Transmissible Spongiform Encephalopathy |
| vCJD | Variant Creutzfeldt-Jakob Disease |
| WB | Western Blot |
| WS | Warning Statement |

## EXECUTIVE SUMMARY

This is the report of an EMEA expert workshop on CJD risk and urine-derived medicinal products that was held in July 2007. A summary of relevant publications since the meeting is included. The report will form a basis for the update of the CHMP Position Statement on CJD and plasma-derived and urine-derived medicinal products (EMEA/CPMP/2879/02/rev 1).

In the three years preceding the workshop, three independent research groups had reported TSE infectivity in urine from scrapie-infected rodents. New data on tissue distribution and infectivity in animals had also become available.

The purpose of this workshop was to review the information from the latest ongoing and published experiments in animals on TSE infectivity in urine, with a special focus on the possible relevance of these experiments to the question of whether CJD infectivity could be present in human urine used for the production of medicinal products. Participants also reviewed the latest CJD epidemiology data and manufacturers of urine-derived medicinal products provided results about the investigation of their manufacturing processes and their capacities to remove/inactivate the CJD agent if present in urine.

The meeting was divided into two main sections: scientific sessions on TSE infectivity in urine and sessions related to epidemiology and CJD risk assessments of urine-derived medicinal products.

Representatives from the main research groups working in the area of CJD/TSE in urine, from regulatory agencies (FDA, Health Canada, Swissmedic in addition to EMEA) and from companies with urine-derived active substances and medicinal products participated in the meeting.

The main conclusions of the meeting were:

1. At the time of the meeting, TSE infectivity in urine had been reported in a hamster model. Infectivity was detected at a low titre (similar to that in plasma) during the clinical phase of the disease.

   There was no experimental evidence to support a direct relationship between the infectivity in urine and infectivity in other tissues (e.g. blood, kidney, urinary bladder). The origin of the infectivity in urine remains unclear.

   The data presented were considered rather convincing but should be confirmed in other animal models.

2. Experiments in scrapie-infected mice with coincident lymphocytic nephritis reported by A. Aguzzi showed that infectivity in urine could be affected by inflammatory processes in the kidney. The results suggested similar urine infectivity titres in the presymptomatic and terminal phases of the disease. There was not enough information on the level of infectivity resulting from nephritic inflammation.

   On the basis of this finding, refinement of donor questionnaires with regard to kidney inflammation (e.g. diabetes, autoimmune diseases) should be considered.

3. On the basis of the available data, no conclusion could be reached on whether there is a relationship between prionuria and proteinuria.

4. There is still no confirmed immunochemical or any other chemical detection of $PrP^{Sc}$ in urine.

5. There is no epidemiological evidence of CJD or vCJD transmission by urine-derived medicinal products. The available epidemiological data are reassuring, although epidemiological data alone do not seem a sufficient basis to confirm the safety of such products. For vCJD, the epidemiological experience is too limited, given the long incubation periods, to reach conclusions on whether or not vCJD could be transmitted by urine-derived medicinal products.

6. Manufacturers are recommended to undertake an evaluation based on published data on the contribution of their manufacturing processes to reduce/eliminate TSE agents, as described in the "CHMP Position statement on CJD and plasma-derived and urine-derived medicinal products" (EMEA/CPMP/BWP/2879/02/rev1). Manufacturers should be encouraged also to conduct investigational studies to evaluate the capacity of their manufacturing processes to

remove/inactivate TSE agents using the stepwise approach already recommended for plasma-derived medicinal products.

Different steps in the production of gonadotrophins were identified as potential efficient steps to remove prions (e.g. nanofiltration, chromatographic steps and alkalinisation steps).

7. Risk assessments of urine-derived medicinal products consider the theoretical prion load, the prion clearance capacity of the manufacturing process and the product yield. They are strongly influenced by estimates of the reduction factor of each manufacturing step, which can be of great variability. In addition, the relevance of data on urine infectivity from animal experiments to the human situation is unknown.

8. From the results obtained on the evaluation of the risk reduction from the manufacturing processes, manufacturers consider that the potential exposure of patients undergoing fertility treatments with urine-derived medicinal products is low and unlikely to result in any iatrogenic transmission of CJD.

   These risk assessments were based on the estimations of the prevalence of sCJD and vCJD in countries where the urine is collected, data available on urine infectivity (taken from the hamster model), the results of the investigational studies from the different manufacturing processes and the maximal quantity of urine used in the manufacture of the products per treatment.

   Prion clearance resulting from the investigation of different manufacturing steps with potential TSE reduction capacity, such as filtration, adsorption, chromatographic steps, or nanofiltration, ranged from 1 to 6 logs.

9. There is a different regulatory approach for these products in the different countries. Some countries request different measures such as CJD related questions in the medical questionnaire to donors or the inclusion of warning statements to cover all transmissible agents in the product information.

10. There is a need to review the CHMP position statement on CJD and plasma and urine-derived medicinal products to include the new information available and the main conclusions of this meeting.

## SUMMARY OF THE WORKSHOP SESSIONS

Declarations of any conflicts of interest of experts were made at the start of the workshop.

## 1. SESSIONS ON TSE INFECTIVITY IN URINE

### PRESENTATIONS

- **Infectivity in urine of hamsters infected with scrapie.**
  **Dr. L. Gregori**

L. Gregori presented the titration results of TSE infectivity of urine from 263K scrapie-infected rodents. Results showed that infectivity is present in urine of scrapie infected hamsters. At the time of the experiments (559 days post inoculation), 17/292 (5.8%) of animals inoculated with urine from infected hamsters were clinically infected vs. 1/40 (2.5%) control group. L. Gregori indicated that the cause of the positive animal in the control group was most likely due to a technical error and further investigations were been carried out. The titres in urine in the clinical phase are estimated as approximately 3.6 ID/ml urine and are similar to the levels calculated for plasma (6 ID/ml) in the same hamster model.

L. Gregori provided results demonstrating that infectivity is present in bladder and kidney of the infected hamsters that had donated urine. Tissue infectivity results at 426 days post inoculation show that the titre of both tissues was approximately $10^5$ $\log_{10}$ $ID_{50}$/g of tissue. This titre is $10^5$-fold lower than that of brain but $10^4$–fold higher than the infectivity of blood. Tissue innervations could be a possible cause of infectivity although additional studies are needed to verify this hypothesis.

Different tissues were considered as possible origin of infectivity in urine such as blood, shedding from kidney and/or bladder, contamination (e.g. from skin, hair, faeces) or inflammation. At the moment, there is no experimental evidence to support any direct relationship (see also Section 5 of this report).

- **'*In vivo*' and '*in vitro*' experiments regarding PrP in urine and TSE infectivity in urine.**
  **Dr. Z. Kariv-Inbal**

Z. Kariv-Inbal referred to the group's published findings of transmission of urine infectivity to hamsters from scrapie-infected hamsters.[1]

She commented that prion urine samples comprise an array of protease resistant proteins including protein light chain immunoglobulin (IgGκ chain). Protease resistant light chain is present in urine of CJD patients and healthy carriers of the PrP E200K mutation. She presented results of an electrophoretic method using tricine gel and a specific antibody (IPC-1) where the signal of PrP can be differentiated from the light chain immunoglobulin. Results suggest the presence of small amounts of PK-resistant PrP in urine of experimentally infected hamsters. In order to support this conclusion, Z. Kariv-Inbal pointed out that it would be necessary to confirm the sequence of the urinary PK resistant PrP.

In addition, the group is investigating experimental autoimmune encephalomyelitis (EAE) in scrapie infected mice to determine whether brain inflammation may affect the clinical outcome. The induction of EAE in mice infected with scrapie brain homogenate resulted in early death of the animals. Clinically co-induced mice suffer from clinical signs of both scrapie and EAE. Studies are ongoing in mice inoculated with plasma, blood cells or urine. Currently data do not allow any conclusion to be reached on whether humans subclinical for prion disease suffer from increased susceptibility to inflammatory insults.

- **Pathogenesis of urinary prions.**
  **Prof. A. Aguzzi**

A. Aguzzi presented the transmission studies performed on mice with chronic aggressive hepatitis (AlbLTαβ), pancreatitis (RIPSLC) or both (RIPLTα). Results using Western Blot, bioassay in *tga20*

mice and Scrapie Cell End Point Assay (SCEPA) methods showed that the $PrP^{Sc}$ accumulates in inflamed kidney and pancreas. $PrP^{Sc}$ could not be identified in non-inflamed kidney and pancreas of mice.[2]

Experiments with scrapie infected mice with pancreatitis and nephritis (RIPLTα) or lupus erythematosis and glomerulonephritis (NZBW) showed prion infectivity in the kidney. The coincident prion infection and lymphocytic nephritis lead to prionuria in mice (infectivity detected by bioassay in mice). Prion infectivity titres were found to be relatively low but consistent in the urine of both models of chronic inflammation. Infectivity was detected already in presymptomatic (60-130 days post-infection) and in terminal scrapie sick animals. All controls were negative.

Experiments also showed that overexpression of $PrP^{C}$ in kidney, inflammation and prion replication in liver or sterile glomerulonephritis are insufficient to induce prionuria. Extrarenal inflammation enables prion accumulation at the site of inflammation but does not induce prionuria. According to the results, intrarrenal organized inflammatory foci are required for prionuria.

A. Aguzzi also described on-going experiments investigating whether infectivity and $PrP^{Sc}$ is found in kidneys and urine of sheep with scrapie. $PrP^{Sc}$ was found in kidney in over 70% (56/72) of terminally scrapie sick sheep (SARDA) and in 12% of subclinically scrapie sick sheep. In some instances the amount of $PrP^{Sc}$ found in kidney reached levels that were approximately 25% of that found in brain. Renal homogenates of scrapie sick sheep were infectious. $PrP^{Sc}$ was also detected in urine by protein misfolding cyclic amplification assay (PMCA) in 9/14 urine samples derived from terminal scrapie sick sheep. Transmission experiments into bank voles are ongoing. At the time of the meeting, all negative controls (urine-derived from sheep that come from scrapie symptom free flocks) were negative.

- **Mass spectrometric characterization of the PrP present in urine. Inoculation of human CJD urine into transgenic mice expressing human PrP.**
  **Prof. P.L. Gambetti**

The presence of $PrP^{C}$ in normal human urine and of $PrP^{Sc}$ in urine from patients with prion diseases is controversial. The group of P. Gambetti has searched for $PrP^{C}$ in normal urine and for PrP infectivity in the urine of patients with sCJDMM1, the most common form of sporadic CJD. His presentation included results of ongoing experiments on characterisation of peptides identified as PrP by mass spectrometry.

Results showed PrP present in urine is mostly in an N-terminally truncated form (18-28 kDa, truncated beyond the 3F4 epitope, at residue 112), which contrasts with PrP in brain where both full length (27-35 kDa) and truncated forms are present. The concentration in urine is approx 10 ng PrP/mL.

The urinary PrP is diglycosylated. Experiments on two-dimensional gel electrophoresis showed that urinary PrP is modified by heterogeneous acidic glycans. At least 10-12 glycoforms of urinary PrP having acidic PIs of 3.8-5.3 could be identified. After treatment with PNGase F, these glycoforms are reduced to 3 more basic deglycosylated isoforms (PIs: 5.4, 5.7 and 6.0).

Detergent studies suggest the presence of an anchor that has lost its lipidic component indicating that PrP in urine may have been shed from cell surfaces.

L. Gambetti commented that they are currently performing experiments to evaluate the prion infectivity of human urine from sCJD in humanized Tg mice. At the time of the meeting (post 487 days) there was no evidence of infection in the mice, suggesting low or no infectivity.

- **Evaluation of urinary $PrP^{Sc}$ as a diagnostic test for sporadic, variant and familial CJD.**
  **J. Ironside**

J. Ironside presented the results on the screening of 100 urine specimens from patients referred to the UK National CJD Surveillance Unit (NCJDSU) as suspected CJD (1991-2003), using the methodology described by Shaked *et al*.[3] for the detection of a protease-resistant prion isoform present in urine.

The presence of urinary $PrP^{Sc}$ as described by Shaked *et al*. could not be confirmed in patient specimens. The method probably detects κ and λ-light chains of immunoglobulins shed in urine. Therefore the test is not informative in the context of clinical CJD surveillance.

- **Infectivity and $PrP^{TSE}$ in the urine of animals with natural and experimental TSE: A Review. R. Bradley**

R Bradley reviewed the available studies regarding infectivity and $PrP^{TSE}$ in urine of animals with natural and experimental TSE. He considered three sources of evidence: epidemiology, transmission in bioassays and biochemical/molecular ($PrP^{TSE}$).

Regarding the epidemiologic point of view, it has been demonstrated that scrapie and CWD are contagious. Identified sources of infection are placenta in sheep (scrapie) and saliva in cervids (CWD). In regard to BSE there is not any evidence of contagion in cattle. There is no epidemiological evidence that urine is involved in natural transmission of TSE infection, although this is theoretically possible for scrapie and CWD. For vCJD, it is too early to reach conclusions from epidemiological evidence.

Regarding transmission via urine, few bioassay studies have been performed (kuru, CJD, BSE, CWD, hamster 263K scrapie). In natural disease no positive findings have been reported. (Regarding CJD there was one single patient study reporting infectivity in urine, whose results have not been confirmed and not accepted by a WHO expert group in 2006). Regarding CWD the study design considered faeces and urine together and only the oral route was considered. Urine from BSE infected cattle was inoculated IC and IP in cattle with a negative outcome.

Only in experimental 263K scrapie in hamsters, a reliable highly sensitive model with no species barrier, is there convincing evidence of infectivity in urine and then only in the terminal stage of disease and at low titre similar to that in plasma. It is not known whether infectivity is present in the preclinical phase.

This may not reflect what happens in natural TSE in humans or animals and especially during the incubation period. Animal to animal transmission from excreted urine is not proved but not excluded.

The possible sources of TSE infection in urine could come from blood, kidney, ureters, urinary bladder, urethra and other parts of the urinary or reproductive tract. Regarding blood, transmission of infectivity by transfusion has been shown in CWD, vCJD, and scrapie and experimental BSE in sheep. Additionally, infectivity in blood has been demonstrated by other routes of inoculation in TME in mink, GSS and CJD in mice, and 263K scrapie in hamsters. In kuru, CJD, TME, kuru in primates, CJD in mice, and scrapie in hamsters, kidney has been demonstrated to be affected. Bladder has been found positive for infectivity in experimental TME and hamster 263K scrapie. There are no studies regarding ureters and urethra.

There is no convincing evidence of $PrP^{TSE}$ in urine or blood. No studies were found that investigated $PrP^{TSE}$ in bladder. $PrP^{TSE}$ has been found in kidney in several natural and experimental TSE.

The main conclusions are that urine can be infective in TSE but the titre is low. There is little evidence of infectivity in natural TSE.

- **Bioassay for infectious prions in body fluids from deer with chronic wasting disease (CWD). Dr. C.K. Mathiason - Teleconference**

C. Mathiason presented her results on CWD transmission in deer.[4] White-tailed deer ingested orally saliva or urine/faeces or received IV/IP transfusion of blood from infectious CWD+ deer. After 24 months of experiments, saliva and blood seemed to be efficient ways of transmission of CWD.

The experiment design assessed both excreted urine and faeces combined. During 18-19 months, all of the animals that ingested faeces and urine remained negative.

It was discussed that these experiments were done by the oral route, which in terms of efficiency of transmission is much less than via IC. These experiments have also the limitation that faeces and urine were analysed together. C. Mathiason indicated that experiments were ongoing to look for urine infectivity using transgenic cervid mice (see section 5 of this report).

- **Detection of $PrP^{Sc}$ in biological fluids.**
  **Dr. C. Soto – Teleconference**

C. Soto presented the experiments of amplification of $PrP^{Sc}$ in biologic fluids by the Protein Misfolding Cyclic Amplification (PMCA) technique. The technique is based on amplification cycles (incubation + sonication). There are ongoing experiments using the product obtained by the amplification cycles to check whether infectivity is maintained during amplification (see also Section 5 of this report on post-meeting publications).

**DISCUSSION SESSION (I) (TSE infectivity in urine, animal data, nature of urine infectivity, tissue infectivity)**

- ***What levels of infectivity are found in urine compared with plasma and infected tissues?***

The reported levels of infectivity in urine seem to be lower than in blood (10 ID/mL) but are in comparable ranges. Experiments in scrapie infected hamsters (L. Gregori) and in lymphocytic nephritic mice (A. Aguzzi) found infectivity titres of 3.4 ID/mL and 1.0 infectious units/2mL respectively. Infectivity had not so far been detected in urine from patients with sporadic CJD (P. Gambetti) or from deer with CWD (C. K. Mathiason).

The data on infectivity in urine is limited and the models used in the experiments are also limited. When interpreting the data, it should be taken into account that: there are no data on whether infectivity is present in the preclinical phase in the scrapie-infected hamster model, the identification of $PrP^{Sc}$ in urine has not been confirmed, and that the reported urine infectivity in a mouse model (at the pre-symptomatic and terminally sick phases) only occurred when there was lymphocytic nephritis.

It was noted that the sensitivity of methods to detect prions in urine should be increased to detect the low concentrations which might be present. The volume of urine produced can vary between infected and non-infected animals and between the preclinical and clinical phase and this may affect the concentration of any infectivity in urine.

It is difficult to identify the most appropriate model to study urine infectivity. The agents causing vCJD and BSE are indistinguishable and yet the diseases are not alike pathologically or clinically. The same BSE strain in cow and human has different tissue distribution and pathophysiology in the two species. When studying urine infectivity, different animal models should be considered.

When analysing the urine infectivity, data are needed from different models using different routes of administration of the inoculum. It is noteworthy that intracerebral administration breaks the blood brain barrier and allows $PrP^{Sc}$ to enter the blood; these conditions are not happening in sCJD. Intraperitoneal or oral administration represents a systemic route more equivalent to vCJD but requires higher doses leading to a likely bias of detecting the inoculum.

The results obtained in the hamster model should be reconfirmed in different models using different routes to administer the inoculum. Infectivity in the preclinical phase and the impact of an inflammatory process on the levels of urine infectivity should also be further investigated.

- ***What is the relationship between proteinuria and prionuria in animals?***

No clear conclusion can be reached from the available data on whether there is a relationship between proteinuria and prionuria and/or urine infectivity.

The results from the laboratory of A. Aguzzi indicated that coincident prion infection and lymphocytic nephritis lead to urine infectivity in mice. These data need to be confirmed in other animal models.

- ***Is infectivity in urine present in the pre-symptomatic phase of the disease? Would it be reasonable to assume that human urine could potentially contain a low level of CJD infectivity?***

Results of experiments in hamsters showed that infectivity is present in the symptomatic phase of the disease. There is a lack of data on whether infectivity is present in the pre-symptomatic phase. In the

experiments from the group of A. Aguzzi, infectivity was detected in the pre-symptomatic phase but only in mice with coincident prion infection and lymphocytic nephritis.

In humans (both the sCJD and vCJD cases) there is an absence of evidence, which does not mean an evidence of absence. A low level of infectivity in urine may not be detected by the current methods.

- ***What information is available to give some indication of the nature of infectivity in urine (e.g. size of $PrP^{Sc}$, PK resistance, physicochemical properties)?***

There are no confirmed data available to give some indication of the molecular nature of infectivity in urine.

Currently there is no experimental evidence to support any direct relationship of the infectivity in urine and infectivity in other tissues (e.g. blood, kidney, urinary bladder). The origin of infectivity in urine remains unclear.

## 2. SESSIONS ON EPIDEMIOLOGY AND CJD RISK ASSESSMENTS

### PRESENTATIONS

- **Update on CJD and vCJD epidemiological data.**
  **Prof. R. Will**

In Australia, five cases of iatrogenic CJD have been reported in recipients of human pituitary-derived gonadotrophins (3 definite, 1 probable, 1 possible). The cases were identified promptly but the clinical phenotype in iCJD due to peripheral exposure is atypical (predominant early cerebellar syndrome) and it was this and the younger age that prompted review of medical history. In the two iCJD cases that were published, the age at onset was 40 and 44 years, with an estimated incubation period of 13 years. The short incubation period is related to exposure to treatments potentially contaminated with brain titres of infectivity. In R. Will's opinion, the incubation period would be extended if cases of CJD related to urinary-derived medicinal products were to occur, and therefore the individuals may not be in the 'younger' age groups at the time of clinical onset. This raises the question of whether iCJD would be identified in recipients of urine-derived products by passive surveillance.

There is no epidemiological evidence of CJD or vCJD transmission by urine-derived medicinal products based on the following:

- No evidence of risk linked to infertility treatment and urinary gonadotrophins particularly as regards to CJD. Only one female vCJD patient in UK had received fertility treatment. The period from the start of the treatment to the onset of symptoms of vCJD (20 months) was not compatible with any causal relationship
- Out of 169 female sCJD patients referred to NCJDSU, no history of treatment of infertility has been reported. Under-reporting of infertility treatment cannot be excluded
- Epidemiological data related to exposures to urine (e.g. families of sCJD patients, nursing staff) do not show any evidence of transmission of infectivity
- The use of gonadotrophins has been increasing constantly since 1970 meanwhile the number of vCJD deaths is decreasing. The sex distribution of sCJD cases remains stable with slightly more female cases than males, which may be related to longer life expectancy in females.

R. Will concluded that the available epidemiological data are reassuring, although epidemiological data alone does not seem a sufficient basis to confirm the safety of such products.

- **Urine-derived medicinal products and CJD risk.**
  **Dr. M. Martin**

M. Martin presented the different aspects to be documented for the assessment of the risk to transmit CJD by urine-derived medicinal products. A set of EMEA guidance dealing with the risk linked to plasma-derived medicinal can be transposed to urine-derived medicinal products.

The "CHMP Position statement on CJD and plasma-derived and urine-derived medicinal products" (EMEA/CPMP/BWP/2879/02/rev1) recommends manufacturers to undertake a theoretical evaluation of the potential for risk reduction of their manufacturing processes. This calculation is based on three key components: the theoretical prion load, the prion clearance capacity and the product yield.

Regarding the theoretical prion load, the risk is linked:

- To the probability of having one CJD-incubating donor in the urine pool, depending on the capacity to exclude some familial and iatrogenic forms by an appropriate donor selection, and depending also on the incidence of sporadic and variant CJD in the collection region
- To the level of infectivity that could be present in urine of CJD-incubating donors; the level of infectivity found by L. Gregori *et al.* in the urine of 263K-infected hamsters may be used as a worst-case
- To the volume of urine per donor.

In the estimation of prion clearance capacity, the stepwise approach adopted for plasma-derived medicinal products and defined in the CHMP position statement on CJD could be followed. Some particular aspects when estimating the clearance capacity should be discussed carefully, such as comparability of the conditions used in investigational studies to the real process conditions. The possibility to add reductions factors to estimate the global clearance capacity should follow rules set up in the Note for Guidance on virus validation studies (CPMP/BWP/268/95). Other aspects more specific to TSE investigational studies should also be considered (CPMP/BWP/5136/03).

Concerning the product yield, the amount of urine used for the production of a yearly dose of the considered medicinal product should be estimated. This will enable a theoretical prion load linked to this dose to be calculated. Finally, the risk of TSE infectivity in the finished product is equal to the amount of infectivity present in the volume of urine necessary to produce one yearly dose divided by the estimated reduction factors. Impact of route of administration may also be discussed.

- **TSE risk assessment of urinary gonadotrophins.**
  **Dr. P. Comer (independent study for Ferring Pharmaceuticals)**

P. Comer presented the analysis of the risk linked to the use of gonadotrophins manufactured by Ferring Pharmaceuticals taking into account the sourcing and the manufacture, and making hypothesis on TSE infectivity in urine, epidemiology of TSEs in Argentina, and by using a model of assessment.

- The sourcing consists in selecting voluntary and unpaid post-menopausal woman in Argentina, average age 65 years.
- Urine is processed by several extraction/precipitation and chromatographic steps, two of which were validated for their capacity to remove TSE infectivity.
- The total dose per treatment cycle follows a log normal distribution.

It was emphasised that no cases of transmission of CJD via urine or urine-derived medicinal products have been reported.

The subcutaneous (SC) route of administration is likely to be less efficient than intracerebral (IC) route particularly for the level of infectivity that could theoretically be present in urine.

As regards the geographical origin of urine collection, Argentina is considered as GBR I, i.e. negligible BSE risk, and the prevalence of sCJD is similar to other countries worldwide.

The risk assessment was based on the following assumptions:

- TSE infectivity is present in urine of person who would later develop sCJD, at a level determined by L. Gregori *et al.* in a hamster model (3.4 ic ID/ml)
- A relative efficiency of SC inoculation to IC varying from zero to 10,000 less efficient, a production process reducing TSE infectivity by 0 or 2 logs
- Considering both the infection efficiency of the route of administration and the likely reduction in TSE infectivity due to the process, the overall exposure to TSE infectivity is likely to be reduced by a factor of 4 logs or greater, and unlikely to be less than 2 logs

- Under this assumption the mean cumulative infectivity exposure for a woman undergoing a treatment requiring the higher quantity of product is estimated to be less than 1 infectious unit.

He concluded that the potential exposures to patients undergoing fertility treatment are relatively low and unlikely to result in any iatrogenic transmissions of CJD.

- **Animal and Human TSEs Situation in Argentina.**
  **Dr. C. Van Gelderen (for Instituto Massone)**

As regards animal TSE, several risk analyses have been made since 1990, regulatory measures on surveillance, reporting and importation were introduced in 1990, and subsequently the regulations as regards the use of animal proteins in ruminant feed were reinforced.

BSE surveillance by brain sampling has been in place since 1999. Since 2000, Argentina has been internationally recognized as being in the lowest risk category.

As regards human TSEs, a CJD referral centre was established in 1983, and a surveillance centre manages case referral/dataset since 1997 following WHO recommendations. In conclusion, sporadic CJD occurs in Argentina at the same incidence as in other countries with comparable surveillance. No cases of vCJD have been recorded in Argentina.

- **A TSE risk analysis of human post-menopausal urine-derived gonadotrophins intended for use in human patients with reduced fertility.**
  **Dr. R. Bradley (independent risk analysis for Instituto Massone)**

In Argentina, the risk is brought by donors incubating CJD i.e. sporadic CJD, some forms of familial CJD and rarely iatrogenic CJD.

Regarding the source of urine, the risk can be managed by selecting voluntary unpaid donors with a view to excluding donors with a TSE risk.

The manufacturing process includes several steps (filtration, adsorption, extraction, chromatographic steps) which are potential TSE reduction steps. The estimated global titre reduction capacity (>3 logs) is under evaluation.

Subcutaneous route of administration is a relatively inefficient route for transmitting TSE.

The safety of gonadotrophins extracted from Argentinean post-menopausal urine relies on the negligible BSE risk (GBR I), the absence of vCJD cases, the selection of donors with no known TSE risk, and the theoretical TSE removal capacity of the manufacturing process. In conclusion, the TSE risk linked to gonadotrophin derived from the urine of post-menopausal Argentine women can be considered as negligible.

- **Prion removal by nanofiltration: validation by Scrapie Cell Assay.**
  **Prof. A. Aguzzi (for IBSA)**

Two steps of the production of gonadotrophins by IBSA were identified as potential efficient steps to remove prions: the alkalinisation step and the nanofiltration step.

The assay used to assess prion titre reduction was the Scrapie Cell End Point Assay (SCEPA, modified version of Klöhn et al., 2003) considered equivalent to bioassay.

The effect of alkalinisation was tested first on $PrP^{Sc}$ levels by semi-quantitative western blot (WB). An average reduction of $PrP^{Sc}$ content of 1.5 logs was achieved. The SCEPA confirmed a reduction in prion infectivity of at least 2 logs.

As regards nanofiltration, the procedure was first validated for the use of an enriched prion preparation allowing a high recovery of infectious prions. Within the sensitivity of the SCEPA a complete removal of infectivity is achieved by nanofiltration (>5 logs).

Overall, the process for the manufacture of gonadotrophin used by IBSA would reduce TSE infectivity by at least 7 logs by two orthogonal procedures.

- **Urine-derived hCG: an assessment on CJD risk.**
  **Dr. M. Wiersma – (API/Biotech – Organon)**

The assessment of the risk of transmitting CJD by human chorionic gonadotropin (hCG) produced by Organon took into account the measures taken at the donor selection level and the capacity of the manufacturing process to reduce prion infectivity.

Urine was collected in Brazil and the Netherlands. The donors were recruited without any payments. In the Netherlands, they filled a questionnaire which includes some of the exclusion criteria mentioned in the CHMP Position statement on CJD June 2004 (i.e. transfusion, neurosurgery, UK residence). Similar questions were asked in Brazil.

The purification process includes some precipitation and adsorption steps. The precipitation step in ethanolic conditions was studied in a down-scaled model and showed a reduction capacity of infectivity above 5 logs measured by immunoblot using brain homogenate from a Hamster-adapted scrapie strain. The comparability with bioassay was made indirectly using data from the literature.

The highest dosage of hCG was equivalent to a maximum of 500 ml urine. There have been 2 cases of vCJD in the Netherlands. The one female case could be traced back as not having donated to the collection programme.

In conclusion, the risk of prion transmission by the use of urinary hCG can be considered as very remote given that the donors are young healthy pregnant woman who are selected by exclusion criteria lowering potential contamination risks, the occurrence of vCJD in sourcing countries is low or absent, and the manufacturing process has proved to be capable of removing potential prion contamination.

**DISCUSSION SESSION (II) (Measures to reduce CJD risk for urine-derived medicinal products and CJD Risk assessments)**

- ***Can epidemiological experience be of assistance to reach a conclusion regarding whether or not CJD or vCJD could be transmitted by urine-derived medicinal products?***

There is not epidemiological evidence of CJD and vCJD transmission by urine-derived medicinal products. The evidence for CJD is reassuring although R. Will has illustrated that epidemiological data alone could not be conclusive. Therefore, other evidence has to be considered. For vCJD, the epidemiological experience is too limited, given the long incubation periods, to reach conclusions on whether or not vCJD could be transmitted.

- ***Which steps of the manufacturing process of urine-derived medicinal products are responsible for the reduction of CJD transmission risk?***

Risk assessments of urine-derived medicinal products are strongly influenced by the reduction factor of each manufacturing step, which can be of great variability.

Small pore nanofiltration seems to be an effective method. However, with nanofiltration the main concern is that the aggregation state of a potential prion contaminant in urine or in the respective manufacturing process intermediate is not known and therefore the relevance of reduction factors measured in spiking experiments cannot be established.

Information in literature on purification steps (e.g. chromatography or precipitation) is available indicating significant prion reduction under conditions close to manufacture of urine-derived medicinal products. It has to be demonstrated whether treatment at pH>11 could possibly reduce infectivity.

- ***Should investigational studies on the manufacturing process of urine-derived medicinal products be requested?***

Further investigational studies on the manufacturing process of urine-derived medicinal products are recommended to be performed. There is a high variability in the reduction capacity of each step depending on the different conditions in the manufacturing processes. Therefore, it is not advisable to

rely only on the efficacy of removal steps from investigational studies for plasma-derived medicinal products.

As for plasma-derived medicinal products, the physicochemical form of the spiking agent can affect the measured reduction capacity. The preconditioning of the spike should be described in detail. It has a great influence on the experimental findings as shown for plasma-derived medicinal products (e.g. treatment with detergents). The importance of the aggregation state has also to be taken into account particularly when investigating nanofiltration. If inactivation processes are investigated (e.g. heat, alkali), the selection of strains of TSE agent should take into account strain differences in resistance to the inactivation process under investigation.

The consistency of the findings should be evaluated. It is important to model the manufacturing process. The different removal steps should be evaluated individually and sequentially, where possible, and the summing up of removal factors should be justified in the discussion. Cell culture assays should be combined with bioassays and the most suitable test should be used.

- ***Should the stepwise approach required for blood-derived products also be required for urine-derived products?***

A stepwise approach should be followed.

- ***Should exclusion criteria for urine donor selection be required for all products?***

Whenever exclusion criteria can be applied, this is highly desirable. Further consideration is needed on whether the same exclusion criteria should be required for all products.

Refinement of questionnaires with regard to kidney inflammation (e.g. diabetes, autoimmune diseases) should be considered. A simple urine test to detect kidney inflammatory conditions would be desirable but would need to be developed.

- ***In terms of risk assessments for urine-derived medicinal products, should differentiation be made between collection of urine in a country that has reported cases of vCJD and in countries that have not?***

Mixed opinions were expressed.

On one side, for plasma-derived medicinal products, the CHMP Position Statement recommends 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. No other country-based exclusions are applied. For countries with similar BSE exposure risk, then differentiation between those that have had clinical cases and those that have not would not appear to be justifiable as it cannot be excluded that there may be preclinical cases.

On the other side, the view was expressed that urine should not be collected from countries with vCJD cases. Additionally, urine should not be collected from countries without CJD surveillance.

- ***Should a specific warning statement on transmissible agents be recommended at the EU level for human urine-derived medicinal products? Should it refer specifically to CJD and vCJD?***

Since urine is a human-derived material there is always a potential risk from emerging, unknown transmissible agents (e.g. viruses). Therefore, the need for a suitably worded general warning statement could be further discussed but it was recognised that finding an appropriate wording would be difficult. Specific reference to CJD and vCJD was not considered necessary on the basis of the current information.

- ***Would a recall of products be justified where a donor is later confirmed as having sporadic, familial or iatrogenic CJD or vCJD?***

The efficacy of this measure would be diminished by the fact that this information often comes late, when batches will mainly have been used. In addition, experience with plasma-derived medicinal products has shown that recall for sCJD has led to product shortages. Whether a recall is justified or

not, depends on the risk assessment of the individual product. The same approach as for plasma-derived medicinal products was suggested (i.e. recall for vCJD).

The main conclusions of this session are that despite the need for confirming the data on infectivity in urine,

- Measures to reduce infectivity have to be used,
- Investigational studies should be done (preconditioning of the spiked sample should be tested),
- Potential accumulation of prions on chromatographic columns or a potential batch to batch contamination due to carry-over of prions should be addressed.

## 3. SESSION ON THE REGULATORY REQUIREMENTS FOR URINE-DERIVED MEDICINAL PRODUCTS IN DIFFERENT COUNTRIES.

### PRESENTATIONS

- **Regulatory requirements for urine-derived medicinal products in the EU.**
  **Mrs. G. Silvester (EMEA)**

CHMP published a Position Statement on CJD and plasma-derived and urine-derived medicinal products, which was last reviewed in June 04. At that time $PrP^{Sc}$ in urine had been reported.

In July 2007 the presence of $PrP^{Sc}$ in urine had not been confirmed, TSE infectivity in urine has been reported in animal experiments and there is still no experimental/epidemiological evidence of transmission of CJD by urine.

The guidance in the Position Statement on donor selection criteria, exclusion of donors and theoretical evaluation of the reduction of infectivity in the manufacturing process may need to be updated.

There is a different approach in the Member States on the warning statements (WS) in the product information of urine-derived medicinal products. For plasma-derived medicinal products, the purpose of a WS has been described in CHMP guidance. It is to give clinically important warnings and precautions for use, inform on the overall effectiveness of the measures for the safety of the product, indicate the remaining potential risk of transmitting infective agents and give specific information on viruses that have been transmitted in the past, where applicable.

France and Italy request WS for all urine-derived medicinal products, whilst a WS is only requested for urokinase products in Germany. In all cases, the WSs contain a general text that covers all transmissible agents and do not specifically mention CJD risk.

G. Silvester also described the information that EMEA had received on the regulation of these products in Japan. The requirements are that donations are unpaid, they should be tested by PCR for HBV, HCV and HIV and that there is record keeping of the pooled urine. Human urine collected in a country where a vCJD case has been confirmed should not be used for the manufacture of human urine-derived products. There are no requirements in terms of WS for urine-derived medicinal products in Japan. As for all biological products, package inserts should include the name of the source material.

- **Canadian Regulatory Approach to Human-Urine-Sourced Biologicals**
  **Dr. A. Ridgway (Canada)**

In Canada, the medical questionnaires to donors address CJD. There are no standard CJD risk related deferrals as used for blood products. The regulatory approach is that applicants are requested to address the clearance of adventitious agents. Although there are no specific requirements to address clearance for TSE agents, the practice is that sponsors evaluate this.

For urine-derived medicinal products there are no specific rules regarding pool sizes and traceability, and no specific rules regarding product recalls if a donor subsequently is identified with CJD are applied. Currently there is no request that a WS statement is included in the labelling of urine-derived medicinal products.

In line with the recent studies in animal models and with transparency for users, Canada is having discussions at an internal level on possible measures, such as the introduction of a risk notification in the product labelling, the revision of donor screening questionnaires and donor follow-up measures and a request to manufacturers for a risk analysis of their manufacturing processes.

*Post meeting note*

A. Ridgway informed that a risk notification request was introduced into product labelling for human urine-derived products marketed in Canada in January 2008. The wording includes: "The drug substance of this drug product is manufactured from human urine. Although the risk is theoretical, and no case of transmission of an infectious agent linked to the use of urine-derived gonadotrophins has ever been identified, the risk of transmitting infectious agents cannot be completely excluded."

- **Requirements for the Authorisation of Urine-derived Products in Switzerland**
  **Dr. C. Berger (Switzerland)**

C. Berger highlighted that taking into account that there are new emerging viruses, there is a risk inherent to all products with raw materials of human origin. When knowledge is incomplete and there is limited or absence of evidence, precautions should be considered.

No transmission through urine has been observed. On the other hand, some hints from science point towards risk. The knowledge of surveillance is considered insufficient and measures have been established mainly for blood products. Therefore the presence of infectivity cannot be totally excluded.

In Switzerland, all urine-derived medicinal products are labelled with a warning statement. An informal English translation of the wording is "The drug substance of this medicinal product is sourced from human urine. Therefore the risk of transmitting infectious agents of known and unknown nature can not be totally excluded."

- **FDA Regulatory Approaches to CJD Risk and Urine-derived Medicinal Products**
  **Dr. G.M. Feldman (USA)**

FDA does not have any published guidance regarding urine-derived medicinal products. There are no requirements for CJD-specific donor restrictions, warning statements in the product information or recall for these products.

## 4. SESSION ON UPDATE ON FUTURE SCREENING TESTS FOR HUMAN TSE

### PRESENTATIONS

**Dr. P. Minor**

Some limitations and difficulties of developing a meaningful screening test were presented:

- The nature of abnormal prion protein is not well defined; it aggregates to form insoluble polymeric chains that are resistant to proteinase
- The presence of $PrP^{TSE}$ is not always correlated with vCJD infectivity. However, as a better biomarker has not yet been identified, $PrP^{TSE}$ is currently the most appropriate marker against which any detection test should be targeted.

He considered possible evaluation criteria for test methods for the detection of the abnormal prion protein ($PrP^{TSE}$) and their application for use in man:

- Assay of human brain and spleen, including samples diluted in plasma
- Assay of blinded and replicate blood samples from scrapie positive and negative samples.

It should be shown that the assay measures something in tissues that are known to be infectious. Sensitivity should be established relative to other available methods such as immunoblotting. Assay of blood samples from scrapie positive and negative samples will show whether the assay will or will not

record a signal consistently in a sample which may be positive and will not in similar samples expected to be negative.

P. Minor presented results from a collaborative study organised by NIBSC to assess new diagnostic tests for $PrP^{TSE}$.

He summarised some of the difficulties hampering the development of vCJD diagnostic reagents: few relevant samples, ethical and practical issues, some assays may not be commercially viable.

Four different detection methods were described:

- Epitope Protection

$PrP^{TSE}$ contains epitopes that are buried within the normal form. They can either be revealed by denaturation and detection with an antibody, or the exposed epitopes can be burned-off chemically and the preserved epitopes recognised by antibody after denaturation.

- Specific Capture Methods

$PrP^{TSE}$ interacts with polyionic ligands more strongly than the normal form so it can be specifically purified from a mixture. Such compounds include pentosan and streptomycin which under the appropriate conditions bind and concentrate prions.

- Specific Detection Methods

Some antibodies and ligands react with sequences exposed on $PrP^{TSE}$.

- Protein Misfolding Cyclic Amplification (PMCA)

$PrP^{TSE}$ amplification is accomplished by repeated ultrasound-induced fragmentation of aggregates, followed by formation of more $PrP^{TSE}$.

*Post-meeting note*

A workshop organised by the European Commission met in October 2007 to consider the addition of vCJD diagnostic assays to Annex II list A of *In vitro* Diagnostics Directive 98/79/EC. The conclusions from the workshop were that this issue is important and concerns all citizens of the EU; the common opinion of the attendees to the workshop was that this kind of diagnostic device would appropriately belong to Annex II list A; if added to list A, there would need to be an appropriate Common Technical Specifications (CTS) drafted, preferably available at the same time that the test is listed; and if the test is not added, it potentially poses a public health issue and could have serious implications for public health authorities.

http://ec.europa.eu/enterprise/medical_devices/events/vcjd_presentations/vcjd_%20workshop_summary.pdf

## 5. POST-MEETING PUBLICATIONS ON URINE INFECTIVITY AND/OR ABNORMAL PRION PROTEIN IN URINE

This section intends to summarise the new data related to infectivity in urine and/or abnormal prion protein in urine published between the meeting at the EMEA in July 2007 and the publication of this report.

### *Urine infectivity*

The final results of the experiments presented by L. Gregori[5] at the meeting were published in September 2008. 18 out of 292 (5.4%) hamsters developed clinical scrapie after intracerebral inoculation with pooled urine collected from 22 hamsters showing clinical signs of 263K scrapie. $PrP^{RES}$ was detected in the brain of these 18 hamsters. The infectivity titre of the urine as calculated from the Poisson distribution was 3.8±0.9 infectious doses/ml. Titration of homogenates of kidney and urinary bladders gave concentrations 20,000 fold greater. Histological and immunohistochemical examination of these tissues did not show indication of inflammatory or other pathologic changes except for occasional deposits of disease-associated prion protein in kidneys. The most likely cause of scrapie development in 1 of the 40 control hamsters was thought to be contamination due to a technical lapse during collection of the urine pools.

Presence of infectious prions has also been reported in the urine and saliva of deer with Chronic Wasting Disease (CWD)[6]. Urine and saliva were collected at the terminal stages of infection. Prion infectivity was detected by bioassay of concentrated, dialyzed urine and saliva in transgenic mice expressing the cervid PrP gene (Tg[CerPrP] mice). In a group of 9 mice inoculated intracerebrally with lyophilized urine, 2 animals developed neurologic disease at 370 and 376 days post inoculation. In addition, $PrP^{CWD}$ was detected in pooled and concentrated urine by PMCA. The concentration of abnormal prion protein in bodily fluids was very low, as indicated by: undetectable $PrP^{CWD}$ levels by traditional assays (western blot, ELISA) and prolonged incubation periods and incomplete TSE attack rates in inoculated Tg(CerPrP) mice (373±3 days in 2 of 9 urine-inoculated mice and 342±109 days in 8 of 9 saliva-inoculated mice). There was no evidence of infection in the negative controls.

***Abnormal prion protein in urine***

Prior to the meeting in July 2007, a protease-resistant isoform of the prion protein was reported once in the urine of scrapie-infected hamsters, BSE-infected cattle, and humans with CJD[3] but later these results were declared as a consequence of cross-reaction of secondary antibodies with either contaminating bacterial proteins[7] or immunoglobulin fragments excreted in urine[8,9,10].

Andrievkaia *et al.*[11] reported in 2008 that a proteinase K-sensitive protein band with a MW of 27–30 kDa in urine samples from scrapie-infected sheep and healthy sheep was visualized after immunoblotting with anti-PrP monoclonal antibodies to a C-terminal part of $PrP^{C}$, but not after immunoblotting with monoclonal antibodies to an N-terminal epitope of $PrP^{C}$ or with secondary antibodies only. The amount of $PrP^{C}$ in the urine of 49 animals (control group: n=16; naturally scrapie-infected group: n=33) was estimated by comparison with known amounts of ovine recombinant PrP in the immunoblot. Background concentration of $PrP^{C}$ in urine was found to be 0–0.16 ng/ml. Seven out of 33 (21%) naturally scrapie-infected animals had an elevated level (0.3–4.7 ng/ml) of $PrP^{C}$ in urine. The origin of $PrP^{C}$ in urine and the reason for the increased level of $PrP^{C}$ in scrapie-infected sheep urine has yet to be explored.

No correlation was found between the urinary $PrP^{C}$ concentration, the total protein concentration in urine samples, and severity of scrapie-associated clinical signs. The authors were unable to detect proteinase K–resistant PrP in scrapie-infected sheep urine samples, possibly as a result of levels being below the limit of detection of this method or the lack of proteinase K–resistant PrP excreted in urine.

Muruyama *et al.* published in June 2007[12] the detection of $PrP^{Sc}$ in urine from TSE infected animals using PMCA. Homogenate of brains from hamsters infected with prion strain Sc237 were injected IC or administered orally. Following oral administration, $PrP^{Sc}$ was present in all buffy coat samples examined and most plasma samples in the symptomatic phase. $PrP^{Sc}$ was excreted in urine for a few days after oral administration and at the terminal disease stage. After intracerebral inoculation, urinary $PrP^{Sc}$ was detected in the symptomatic and terminal stages of disease.

In August 08, C. Soto's group published[13] the detection of prions in urine of scrapie infected Syrian Golden Hamsters. Urine was collected from 5 scrapie sick animals intraperitoneally infected by the hamster strain Hyper (HY) as well as control uninfected animals of similar age. $PrP^{Sc}$ in urine was detected in 4 out of 5 animals studied (80%) using the PMCA technique. It was not detected in any of the 5 negative controls. Semi-quantitative calculations suggest that $PrP^{Sc}$ concentration in urine is around 10-fold lower than in blood. This is based on the fact that the $PrP^{Sc}$ was detected after 6 serial rounds of PMCA. $PrP^{Sc}$ maintains its infectious properties, since injection of the amplified agent from this fluid produced a disease indistinguishable from the one induced by non-amplified infected brain material.

In 2008, Dabaghian *et al*[14] reported results of a western blotting analysis on concentrated urine samples of CJD and other neurodegenerative disease affected individuals. Using anti-PrP-antibodies, they detected PK resistant bands in several samples of urine from sCJD and other neurodegenerative disorders patients, which did not appear in PK-treated healthy control samples. They suggest that the bands could be a PK-resistant complex formed by interaction between PrP and immunoglobulin proteins.

### *$PrP^d$ in kidney*

After examination of kidneys collected post-mortem from sheep, naturally or experimentally infected with scrapie, Siso *et al.*[15] reported that disease associated prion protein ($PrP^d$) accumulates in a significant proportion 44% and 51% of kidneys of naturally and experimentally scrapie infected sheep respectively. $PrP^d$ detection was performed by WB and ultrastructural examination.

$PrP^d$ was shown to accumulate in the interstitium of the renal papillae, in association with the cell membrane and lysosomes of fibroblast-like cells, or extracellularly, in close contact with collagen and basal membranes. These deposits were unrelated to inflammatory changes in the kidney as shown by routine histology and by immunohistochemical examination for different immune cell markers. $PrP^d$ accumulated in the kidney of sheep that showed widespread $PrP^d$ deposition in the lymphoreticular system and had long incubation periods; these findings argue for a haematogenous origin of renal $PrP^d$, although the precise site and mechanism (glomerular filtration and reabsorption at Henle's loop, or extravasation from vasa recta capillaries, or both) by which $PrP^d$ leaves the blood to accumulate in the interstitium of renal papillae remain to be determined.

## 6. BIBLIOGRAPHY


1- Kariv-Inbal Z, Ben-Hur T, Grigoriadis NC, Engelstein R, Gabizon R. Urine from scrapie-infected hamsters comprises low levels of prion infectivity. *Neurodegener Dis.* 2006;3(3):123-8

2- Seeger H, Heikenwalder M, Zeller N *et al*. Coincident scrapie infection and nephritis lead to urinary prion excretion. *Science*. 2005 Oct 14;310(5746):324-6.

3- Shaked GM, Shaked Y, Kariv-Inbal Z, *et al*.: 2001, A protease-resistant prion protein isoform is present in urine of animals and humans affected with prion diseases. *J Biol Chem* 276:31479–31482.

4- Mathiason CK, Powers JG, Dahmes SJ, *et al*. Infectious Prions in the Saliva and Blood of Deer with Chronic Wasting Disease. *Science*. 2006 Oct 6;314(5796):133-6

5- Gregori L, Kovacs GG, Alexeeva I, *et al*.: Excretion of transmissible spongiform encephalopathy infectivity in urine. *Emerg Infect Dis*. 2008 Sep;14(9):1406-12.

6- Haley NJ, Seelig DM, Zabel MD, *et al*.: Detection of CWD prions in urine and saliva of deer by transgenic mouse bioassay. *PLoS ONE*. 2009;4(3):e4848. Epub 2009 Mar 18.

7- Furukawa H, Doh-ura K, Okuwaki R, *et al*.: A pitfall in diagnosis of human prion diseases using detection of protease resistant prion protein in urine. Contamination with bacterial outer membrane proteins. *J Biol Chem* 2004, 279:23661–23667.

8- Head MW, Kouverianou E, Taylor L, *et al*.: Evaluation of urinary PrPSc as a diagnostic test for sporadic, variant, and familial CJD. *Neurology* 2005, 64:1794–1796.

9- Kariv-Inbal Z, Halimi M, Dayan Y, *et al*.: Characterization of light chain immunoglobulin in urine from animals and humans infected with prion diseases. *J Neuroimmunol* 2005, 162:12–18.

10- Seeger H, Heikenwalder M, Zeller N, *et al*.: Coincident scrapie infection and nephritis lead to urinary prion excretion. *Science* 2005, 310:324–326.

11- Andrievskaia O, Algire J, Balachandran A, Nielsen K. Prion protein in sheep urine. *J Vet Diagn Invest.* 2008 Mar;20(2):141-6.

12- Murayama Y, Yoshioka M, Okada H, *et al*.: Urinary excretion and blood level of prions in scrapie-infected hamsters. *J Gen Virol*. 2007 Oct;88(Pt 10):2890-8.

13- Gonzalez-Romero D, Barria MA, Leon P, *et al*.: Detection of infectious prions in urine. *FEBS Lett.* 2008 Sep 22;582(21-22):3161-6. Epub 2008 Aug 13.

14- Dabaghian R, Zerr I, Heinemann U, Zanusso G. Detection of proteinase K resistant proteins in the urine of patients with Creutzfeldt-Jakob and other neurodegenerative diseases. *Prion.* 2008 Oct;2(4):170-8.

15- Sisó S, Jeffrey M, Steele P, McGovern G, *et al*.: Occurrence and cellular localization of PrPd in kidneys of scrapie-affected sheep in the absence of inflammation. *J Pathol.* 2008 Jun;215(2):126-34.

## LIST OF PARTICIPANTS

| NAME | ORGANISATION | COUNTRY |
|---|---|---|
| **INVITED EXPERTS / SPEAKERS** | | |
| **Dr. Adriano Aguzzi** | Institute of Neuropathology<br>University Hospital of Zurich | SWITZERLAND |
| **Dr. Ray Bradley** | Private BSE consultant | UNITED KINGDOM |
| **Dr. Zehavit Kariv-Inbal** | Hebrew University, Department of Neurology,<br>Hadassah University Hospital | ISRAEL |
| **Dr. Pierluigi Gambetti** | Case Western Reserve University | U.S.A. |
| **Dr. Luisa Gregori** | Molecular Neurovirology Laboratory<br>Veterans Affairs Medical Center<br>School of Medicine. University of Maryland | U.S.A. |
| **Dr. Candace K. Mathiason**<br>Via teleconference | Department of Microbiology, Immunology, and Pathology,<br>College of Veterinary Medicine and Biological Sciences (CVMBS),<br>Colorado State University (CSU) | U.S.A. |
| **Dr. Robert G. Rohwer** | Molecular Neurovirology Laboratory<br>Veterans Affairs Medical Center<br>School of Medicine. University of Maryland | U.S.A. |
| **Dr. Claudio Soto**<br>Via teleconference | Protein Misfolding Disorders Laboratory, George and Cynthia Mitchell Center for Neurodegenerative Diseases,<br>Departments of Neurology, Neuroscience and Cell Biology and Biochemistry and Molecular Biology,<br>University of Texas | U.S.A. |
| **Prof. Robert Will** | Western General Hospital<br>National CJD Surveillance Unit | UNITED KINGDOM |
| **Dr. Philip Comer** | DNV | UNITED KINGDOM |
| **TSE EXPERT GROUP** | | |
| **Prof. Jean-Hugues Trouvin** (Chairperson) | Agence Française de Sécurité Sanitaire des Produits de Santé | FRANCE |
| **Dr. Christiane Nabein** | Bundesinstitut für Arzneimittel und Medizinprodukte | GERMANY |
| **Dr. Johannes Blümel** | Paul-Ehrlich-Institut | GERMANY |
| **Dr. Vincent Irwin** | Bord Leigheasra na hÉireann | IRELAND |
| **Dr. Marc Martin** | Agence Française de Sécurité Sanitaire des Produits de Santé | FRANCE |
| **Dr. Phil Minor** | NIBSC | UNITED KINGDOM |
| **Prof. Maurizio Pocchiari** | Instituto Superiore di Sanità | ITALY |
| **Dr. Sol Ruiz** | Agencia Española del Medicamento y Productos Sanitarios | SPAIN |
| **Dr. Adrian Thomas** | Medicines and Healthcare products Regulatory Agency | UNITED KINGDOM |
| **CO-OPTED EXPERTS** | | |
| **Dr. Annette Baeckmann** | Bundesinstitut für Arzneimittel und Medizinprodukte | GERMANY |
| **Dr. James Ironside** | University of Edinburgh<br>National Creutzfeldt-Jakob Disease - Surveillance Unit<br>The Bryan Matthew Building<br>Western General Hospital | UNITED KINGDOM |
| **Prof. Donald J. Jeffries** | St Bartholomews Hospital Medical college | UNITED KINGDOM |
| **Dr. Michael Beekes** | P24- Transmissible Spongiform Encephalopathies<br>Robert Koch-Institut | GERMANY |
| **Dr. Jean Manson** | Neuropathogenesis Unit, Roslin Institute | UNITED KINGDOM |

| | | |
|---|---|---|
| **BWP MEMBERS + EXPERTS** | | |
| **Ms. Lucie Benešová** | State Institute for Drug Control | CZECH REPUBLIC |
| **Dr. Marcos Timón** | Agencia Española del Medicamento y Productos Sanitarios | SPAIN |
| **Dr. Miguel Calero** | Agencia Española del Medicamento y Productos Sanitarios | SPAIN |
| **Dr. Birgit Schmauser** | Bundesinstitut für Arzneimittel und Medizinprodukte | GERMANY |
| **Dr. Kristina Kluge** | Bundesinstitut für Arzneimittel und Medizinprodukte | GERMANY |
| **Dr. Keith Watson** | Medicines and Healthcare products Regulatory Agency | UNITED KINGDOM |
| **Dr. Margarida Menezes-Ferreira** | Instituto Nacional da Farmácia e do Medicamento (INFARMED) | PORTUGAL |
| **INDUSTRY** | | |
| **Dr. Paola Ricci** | Europabio | SWITZERLAND |
| **Dr. Lutz Seidel** | Medac | GERMANY |
| **Dr. Peter McAnulty** | Ferring Pharmaceuticals A/S | DENMARK |
| **Mr. Martin Hedenfalk** | Ferring Pharmaceuticals A/S | DENMARK |
| **Mr. Claudio Wolfenson** | Instituto Massone S.A. | ARGENTINA |
| **Mr. José Groisman** | Instituto Massone S.A. | ARGENTINA |
| **Dr. Aliza Eshkol** | Merck Serono GmbH | SWITZERLAND |
| **Herr Jürgen Malinka** | Pharma-Zentrale | GERMANY |
| **Dr. Maurizio Dattilo** | Institut Biochimique SA | SWITZERLAND |
| **Dr. Paolo Caccia** | Institut Biochimique SA | SWITZERLAND |
| **Mr. Francesc Muntañá** | Farma Lepori (Angelini) | SPAIN |
| **Mrs. Dr. Anneke Koole** | Organon | THE NETHERLANDS |
| **Dr. Frans van Houdenhoven** | Diosynth - Organon | THE NETHERLANDS |
| **Dr. Marten Wiersma** | N.V. Organon | THE NETHERLANDS |
| **INTERNATIONAL ORGANISATIONS AND REGULATORY BODIES** | | |
| **Dr. Anna Padilla** | WHO | SWITZERLAND |
| **Dr. Gerald M. Feldman, Ph.D.** | FDA | U.S.A. |
| **Dr. Anthony Ridgway** | CANADA | CANADA |
| **Dr. Christopher Berger** | SWITZERLAND | SWITZERLAND |
| **Dr. Carlos José van Gelderen** | IICA | ARGENTINA |
| **EMEA SECRETARIAT** | | |
| Mrs. Glenda Silvester | | |
| Dr. Alberto Gañán Jiménez | | |
| Dr. Silvia Domingo | | |
| Dr. Brigitte Neugebauer | | |
| Dr. Nick Gate | | |
| Dr. Ana Trullas Jimeno | | |
| Dr. Ragini Shivji | | |
| Ms. Audrey Sultana | | |
| Ms. Charlotta Gustafsson | | |
| Ms. José Childs | | |
| Ms. Kate Balzan | | |

# EXPERT WORKSHOP ON CJD AND URINE-DERIVED MEDICINAL PRODUCTS – EMEA – Room 3A

## WEDNESDAY 11th JULY

| | | |
|---|---|---|
| **16.00** | **PREPARATORY SESSION** | |
| | CJD Expert Group | |
| | M. Martin | 20 mins |

## THURSDAY 12th JULY

| | | |
|---|---|---|
| **09.00** | **INTRODUCTION** | 15 mins |
| **09.15** | **TSE INFECTIVIY IN URINE (I)** | |
| | L. Gregori | 30 mins |
| | Z. Kariv-Inbal | 20 mins |
| | A. Aguzzi | 20 mins |
| | P.L. Gambetti | 20 mins |
| | Questions | 15 mins |
| 11.00 | Coffee | |
| **11.15** | **EPIDEMIOLOGY AND RISK ASSESSMENTS** | |
| | R. Will | 30 mins |
| | M. Martin | 20 mins |
| | P. Comer / Ferring rep. | 20 mins |
| | R. Bradley / Massone rep. | 20 mins |
| | Questions | 15 mins |
| 13.00 | Lunch | |
| **14.00** | A. Aguzzi / IBSA rep. | 20 mins |
| | Organon | 20 mins |
| | Questions | 20 mins |
| **15.00** | **TSE INFECTIVIY IN URINE (II)** | |
| | J. Ironside | 10 mins |
| | R. Bradley | 20 mins |
| | Questions | 15 mins |
| 15.45 | Coffee | |
| **16.00** | C. Soto | 20 mins |
| | C. Mathiason | 20 mins |
| | Questions | 20 mins |
| **17.00** | **REGULATORY SESSION** | |
| 18.00 | BRIEFING WITH MANUFACTURERS | |

## FRIDAY 13th JULY

| | | |
|---|---|---|
| **08.45** | **DISCUSSION PANEL (I)** | |
| | TSE INFECTIVITY SESSIONS | |
| 10.30 | Coffee | |
| **10:45** | **DISCUSSION PANEL (II)** | |
| | EPIDEMIOLOGY AND RISK ASSESSMENTS | |
| **12.30** | **UPDATE ON SCREENING TESTS** | |
| | P. Minor | 20 mins |
| **13.00** | **END OF THE MEETING** | |
| 13.05 | Lunch | |
| 13:35 | RESTRICTED SESSION | |