ISSUE

1. To present the key findings of the Factors Affecting the Evolution of Prion Diseases in the Environment (FATEPriDE) project that examined the possible influence of environmental trace elements on the occurrence of Transmissible Spongiform Encephalopathies (TSEs).

BACKGROUND

2. FATEPriDE is a large recently completed European Union funded project to investigate whether environmental factors may influence the occurrence of TSEs. The main emphasis of the study was the possible role played by trace elements and in particular the balance between manganese (Mn) and copper (Cu). This hypothesis was developed from observations that ions of both elements may bind and influence the structure of the prion protein (PrP) and that there are geographical variations in the concentrations of these elements in the environment and in the occurrence of TSEs. Research also examined the possible role of organophosphate pesticides, which have been suggested by some as possibly involved in the aetiology of Bovine Spongiform Encephalopathy (BSE).

3. The driving force of the project was to bring together both biochemical and geochemical expertise to try to advance understanding of TSEs and environmental factors. Experimental and field studies were conducted by a consortium of eight European research centres. The main aims of the project were:

- Determine any role for organophosphates in the occurrence of TSEs.
- Characterise Cu and Mn binding to PrP in terms of affinity, structural changes and role in protein aggregation.
- Determine the relationship of Cu and Mn to the cell biology of PrP and TSE infection.
• Use animal models to determine the relationship between levels of PrP expression or TSE pathology/disease progression and level of metals in diets.
• Acquire high definition geochemical maps of trace element concentrations throughout UK and Europe to compare to similar maps of TSE incidence.
• Sample specific areas of high TSE incidence to verify suggestions that TSE hot spots are found in regions of high Mn concentrations.

4. A report of the project is in preparation and few of the results from the project have been published. The key findings are summarised below. Professor David Brown, a member of SEAC and the FATEPriDE consortium, will present an overview of the project at SEAC 97.

KEY FINDINGS

5. The findings of most relevance to the main hypothesis are outlined below. However, the outcome of the study included a large number of other findings.

Organophosphate Studies

6. Studies using phosmet (an organophosphate pesticide) were carried out throughout the project. No relationship between this compound and the potential to cause a TSE were identified. In studies with oral dosing of rats, it was shown that PrP expression levels increased in the brain but there was no association between this and formation of proteinase K (PK) resistant PrP.

Studies with Recombinant Protein

7. As Cu ion (Cu^{2+}) binding to PrP had been previously established, Mn ion (Mn^{2+}) binding to PrP was investigated using isothermal titration calorimetry (ITC), which measures thermodynamic changes produced by molecular binding. Titration experiments using purified mouse PrP expressed from bacteria, suggested that two Mn^{2+} ions bound to one PrP molecule. Further titrations using sequence deletion and substitution mutants of PrP suggested a higher affinity Mn^{2+} binding site involving the histidines at positions 95 and 110 and a lower affinity Mn^{2+} binding site within the octapeptide repeat region of PrP. The affinity values obtained were well within the range expected from known Mn^{2+} binding proteins.
8. Further ITC studies showed that Mn$^{2+}$ can bind to PrP even when Cu$^{2+}$ is already present. Binding of Mn$^{2+}$ to PrP at the high affinity site results in conformational changes in the protein and increased PK resistance.

9. The location of Cu$^{2+}$ and Mn$^{2+}$ binding sites on PrP was also investigated using surface plasmon resonance to measure the energy changes produced when PrP and PrP deletion/substitution mutants bound to Cu$^{2+}$ or Mn$^{2+}$. Based on these experiments, it was concluded that Cu$^{2+}$ binds to a site within the octapeptide repeat region and another less well defined site that involves histidines at positions 95 and 110. Mn$^{2+}$ binding site could not be precisely defined but binding was facilitated by the octapeptide repeat region as well as other undefined amino acids, however, in contrast to the ITC analysis, binding was not facilitated by histidines at position 95 and 110.

10. The structure of a truncated form of PrP with or without bound Cu$^{2+}$ or Mn$^{2+}$ was investigated by molecular dynamics modelling. Metal ion binding altered the structure of the truncated PrP and exchange of bound Cu$^{2+}$ with Mn$^{2+}$ also induced structural change in the truncated PrP. Modelling of the truncated form of PrP to clay surfaces was also examined. This suggested there may be strong binding contacts between PrP and clay surfaces.

11. Raman spectroscopy of PrP bound to Cu$^{2+}$ and Mn$^{2+}$ suggested differences in structure depending on the metal ion bound.

12. A model of seed protein aggregation and fibril formation was established using PrP charged with Mn$^{2+}$. PrP-Mn$^{2+}$ was found to form small circular aggregates able to catalyse further protein aggregation and fibrilisation of PrP. This model unlike other published models (for example those of Baskakov et al.\textsuperscript{1}) does not require the presence of denaturants and is not an autocatalytic process (i.e. the substrate of the reaction did not aggregate). The results suggest that Mn$^{2+}$ may play a role in the formation of prion seeds although further studies showed that this material was not infectious in mouse bioassay.

**Cell Studies**

13. The effect of metal binding on the resistance of PrP to PK digestion was investigated using enzyme based assays. In yeast cells expressing mouse PrP, supplementation of the cell culture media

\textsuperscript{1} Baskakov et al. (2002) Pathway complexity of prion protein assembly into amyloid. J. Biol. Chem. 277, 21140-21148.
with \( \text{Cu}^{2+} \) and/or \( \text{Mn}^{2+} \) enhanced the resistance of PrP to PK digestion. This work confirms previous suggestions that high \( \text{Mn}^{2+} \) levels cause cells to form PK resistant PrP.

14. Studies with a scrapie infected cell line (SMB), showed that infection results in a dramatic increase in \( \text{Mn}^{2+} \) concentrations and an increased capacity to retain \( \text{Mn}^{2+} \) within cells. \( \text{Mn}^{2+} \) supplementation increased the levels of PK resistant PrP expressed by SMB cells. Additionally, the presence of increased \( \text{Mn}^{2+} \) concentrations during the infection process resulted in higher levels of PK resistant protein in the infected cells.

15. Analysis of \( \text{Mn}^{2+} \) transporting proteins indicated that the \( \text{Mn}^{2+} \) transporting protein DMT-1 (NRAMP-2) is decreased in classical scrapie infected cells. This is possibly a response from infected cells to prevent further \( \text{Mn}^{2+} \) uptake.

**Animal Studies**

16. The effect of alterations of dietary Cu and Mn levels on the expression of PrP was assessed. Acute changes in dietary Cu or Mn resulted in changes in the level of expression of PrP in the brain. In particular, reduction of Cu in the diet resulted in a decrease in PrP expression while increased Mn with or without alteration in Cu in the diet resulted in a dramatic increase in PrP in the brain.

17. The effects of dietary Cu or Mn supplementation were studied using wild-type mice and three transgenic mouse strains (PrP-knockout, PrP-overexpressing and expressing a form of PrP lacking the octameric repeat region, C4). In particular, increased dietary Mn concentrations resulted in higher levels of Mn in tissues of PrP-overexpressing mice. Wild-type and C4 mice also showed higher levels of Mn in the brain when compared to PrP-knockout mice.

18. The effects of altered metal diets on mice challenged with mouse adapted scrapie were also undertaken. Increased Cu, but not Mn, in the diet extended the incubation period of disease in wild-type mice. In contrast, increased Cu in the diet decreased the incubation period in C4 mice.

19. Blood metal concentrations were determined in sheep experimentally challenged with classical scrapie. The blood concentrations of Mn only were found to increase during the preclinical phase of the disease in line with previous findings from
classical scrapie infected mice. However, Mn increases were also observed in classical scrapie challenged sheep with the most resistant ARR/ARR genotype suggesting the Mn changes occur in response to the challenge rather than the progression of the disease.

Environmental studies

20. Data on trace element concentrations in soil in a number of European locations were used together with geostatistical modelling techniques to produce maps of the geographical distribution and concentration of Cu, Mn and other trace elements as well as the pH and organic content of soils across Europe. No equivalent data for the location of TSEs across Europe could be obtained. Some attempts to compare rough data obtained from the DEFRA website were used in pilot studies but the data were too limited to allow any reasonable conclusion to be drawn for or against the hypothesis of a correlation between areas of high local metal concentrations and rates of TSEs.

21. Simple comparisons of total Mn concentrations in areas of high TSE incidences are unlikely to be meaningful. Two factors need to be considered for any future investigations. The first of these is the bioavailability of Mn in particular areas. Bioavailable Mn is the only form that can be taken up by animals and its concentrations are unrelated to the total Mn in a particular area. Second, variability in Mn absorption by an animal is not principally through absorption by the gastrointestinal tract but is largely through absorption by the lungs. Therefore, estimates of Mn levels need to be assessed in terms of the amounts present in an airborne, bioavailable form along side any other analysis.

22. An examination of classical scrapie risk and trace elements in soil was conducted at a number of sheep farms in Iceland, Italy and France. It was concluded that there was a clear and definite link between areas of classical scrapie susceptibility and higher than usual bioavailable manganese. In addition, decreased levels of copper were also noted in areas of classical scrapie susceptibility (see Annex 1).

SUMMARY

23. The project confirmed and expanded on many previous findings in terms of the biology of PrP and the role of Cu and Mn. These included:
• PrP binds Mn with a reasonable affinity.
• Mn causes the formation of PK resistant isolated recombinant protein or protein in cells.
• Classical scrapie infection increases Mn in cells and in the blood of classical scrapie challenged sheep.
• Mn increases susceptibility of cells to classical scrapie infection.
• Mn binding to PrP produces a form of PrP more resistant to proteinase K and capable catalysing the aggregation of PrP in vitro.

24. The project also generated information concerning the relation of TSEs to environmental factors:

• Potentially no role for organophosphates in TSEs.
• Increased Mn in the diet results in higher PrP levels in the brain.
• No conclusion is yet possible in terms of the relationship between environmental trace element concentrations and the geographical occurrence of TSEs (classical scrapie or BSE).
• Some confirmation was provided that in some specific farms occurrence of classical scrapie correlates with high Mn levels.

ADVICE SOUGHT FROM THE COMMITTEE

25. The committee is requested to note, and may wish to comment on, the findings of the project.

*Provided in confidence*

Roman-Ross *et al.* Exposure to soil high Mn and low Cu concentrations and the development of scrapie. *Unpublished*