Ecosystems supporting clusters of sporadic TSEs demonstrate excesses of the radical-generating divalent cation manganese and deficiencies of antioxidant cofactors Cu, Se, Fe, Zn

Does a foreign cation substitution at prion protein’s Cu domain initiate TSE?

M. Purdey
Taunton, UK

Summary Analyses of food chains supporting isolated clusters of sporadic TSEs (CWD in N Colorado, scrapie in Iceland, CJD in Slovakia) demonstrate a consistent 2 1/2+ fold greater concentration of the pro-oxidant divalent cation, manganese (Mn), in relation to normal levels recorded in adjoining TSE-free localities. Deficiencies of the antioxidant co-factors Cu/Se/Zn/Fe and Mg, P and Na were also consistently recorded in TSE foodchains. Similarities between the clinical/pathological profile of TSEs and Mn delayed psycho-neurotoxicity in miners are cited, and a novel theory generated which suggests that sporadic TSE results from early life dependence of TSE susceptible genotypes on ecosystems characterised by this specific pattern of mineral imbalance. Low Cu/Fe induces an excessive absorption of Mn in ruminants and an increased oxidation of Mn2+ into its pro-oxidant species, Mn3+, which accumulates in mitochondria of CNS astrocytes in Mn SOD deficient genotypes. Deficiencies of scavenger co-factors Cu/Zn/Se/Fe in the CNS permits Mn3+ initiated chain reactions of auto-oxidant mediated neuronal degeneration to proliferate, which, in turn, up-regulates the expression of the Cu-metalloprotein, prion protein (PrP). Once the rate of PrP turnover and its demand for Cu exceeds the already depleted supply of Cu within the CNS, PrP can no longer bind sufficient Cu to maintain its conformation. Mn3+ substitutes at the vacated Cu domain on PrP, thus priming up a latent capacity for lethal auto-oxidative activity to be carried along with PrP like a ‘trojan horse’; where Mn 3+ serves as the integral ‘infectious’ transmissible component of the misfolded PrP-cation complex. The Mn overactivation of concanavalin A binding to glycoprotein and Mn-initiated autoxidation results in a diverse pathological profile involving receptor capping, aggregation/modification of CNS membrane/cytoskeletal proteins. TSE ensues. The BSE/nv CJD strain entails a ‘synthetic’ induction of the same CNS mineral disturbance, where ‘in utero’ exposure to Cu-chelating insecticides/Mn supplements accelerates the onset of a more virulent ‘strain’ of adolescent TSE. © 2000 Harcourt Publishers Ltd

INTRODUCTION

It has been previously suggested that high levels of cation metals such as manganese may interact with the CNS prion protein (PrP) and play a role in the pathogenesis of...
sporadic forms of transmissible spongiform encephalopathy (TSE) (1,2).

Analyses of ecosystems supporting isolated, well defined clusters of sporadic TSE in Iceland, Colorado and Slovakia demonstrate high concentrations of the free radical generating divalent cation, manganese (Mn), as well as marked deficiencies of the radical scavenger enzyme cofactors; Cu, Zn, Fe, Se and the elements Mg/P/Na.

The findings of high levels of Mn in TSE ecosystems may not necessarily indicate any direct relationship between Mn overloading and the aetiology of TSE. It could merely reflect some indirect association between the two; eg, where a further ‘third party’ common environmental factor which promotes the accumulation of Mn in plant material may also promote the survival of ‘agent X’ which causes TSE.

However, various other research studies indicate that the results of this survey may indeed demonstrate a direct aetiological association between Mn exposure and TSEs. For instance, close similarities exist between the pathological/clinical profile of TSEs and the Mn induced delayed psycho-neurodegenerative syndrome found in Mn miners (3,4) where the key idiosyncratic CNS pathological features of TSE (5), such as the presence of amyloid plaques and fibrils, are common to both conditions (6).

Furthermore, 1999 pilot studies conducted by Dr David Brown of the Dept of Biochemistry, Cambridge University, UK, have demonstrated that the divalent cations, Mn and Nickel, will both bind to recombinant PrP and refold the protein into a protease resistant, misfolded isoform. Protease-resistant PrP represents the foremost key feature that hallmarks the pathology of TSE diseased brain (5).

A novel aetiological hypothesis for TSEs is compiled from the data amassed in this pioneer study of TSE ecosystems. It proposes that the trivalent species of Mn cation serves as the all important ‘infectious’ transmissible agent in TSEs.

Mn is a divalent cation, and, much like other divalent species such as Cu and Fe, Mn can perform a dual role in biological systems; as a pro oxidant when freely ‘available’ at high concentrations and as an antioxidant when conjugated onto its respective scavenger enzyme, Mn superoxide dismutase (Mn SOD) (3,4). During the resting stages of the enzymic cycle, MnSOD acts as a safe ‘depot’ for Mn3+, thus protecting tissues against the potential onslaught of its pro-oxidant activities (3,4).

It is proposed that sporadic TSEs will develop in defective MnSOD/PrP genotypes who are chronically dependent on foodchains that are characterised by two abnormal coexisting factors; high levels of the divalent cation Mn and deficiencies of other metals Cu/Zn/Fe/Se which serve as scavenger enzyme co factors in biological systems. (NB; Cu, Fe and P deficiencies in the external food chain would also promote the excessive absorption of Mn across the gut barrier (3,4).

Cu deficient ecosystems generally conform to a seasonal Cu cycle that is characterised by a ten month period of Cu-starvation in vegetation, followed by an ephemeral 20 fold burst of Cu availability during August (8), which, in turn, mediates an ephemeral rise in levels of the Cu-glycoprotein ‘ceruloplasmin’ in mammals thralling off these foodchains (8). Such an abrupt increase in ceruloplasmin turn over could subsequently oxidise a greater proportion of the excess of Mn 2+ into its lethal Mn3+ species (7) – particularly likely to occur in ecosystems deficient in ceruloplasmin’s normal oxidative target, Fe2+. Mn 2+/Mn 3+ species are recognized to concentrate exclusively within the CNS during contexts of Mn overloading (7), where excesses of ‘available’ Mn2+/Mn3+ can persist for up to a year (7). CNS Mn accumulation can result from intranasal intake of airborne concentrations of Mn transported via the olfactory neurones (9) as well as the gastrointestinal route (3,4,7). Genotypes deficient in MnSOD activity would be less able to provide ‘sink’ storage for the excess of Mn, and would therefore be more susceptible to the ravages of Mn 3+ induced chain reactions of oxidant-mediated neurodegeneration (7,10,11); particularly prone to proliferation whilst supplies of Cu, Fe, Zn, Se within the CNS are depleted – metals required as co factors in the major radical scavenger enzyme/antioxidant groups; superoxide dismutases 1/2 (SOD 1/2), glutathione peroxidase, catalases and the antioxidant vitamin E (10).

TSEs and the misfolded metallo-glycoprotein, prion protein

Metalloproteins require specific complementary metals which assist in their folding, catalytic and/or metabolic activities (12). Alternative ‘foreign’ metals can sometimes substitute for the correct metal partner during periods of deficiency. For instance, Mn 2+ or Fe 2+ can also ligate onto the nitrogen of the histidine imidazole rings of certain Cu metalloproteins (12), resulting in the formation of misfolded isoforms which can no longer perform their correct metabolic functions.

TSEs are considered to result from the accumulation of an abnormal protease resistant misfolded isoform of a nerve membrane metalloglycoprotein called prion protein (PrP) (5). PrP is largely expressed in the CNS and lymphatic systems (5).

It is proposed that TSE pathogenesis is initiated (Fig. 1) once Mn 2+ or Mn 3+ substitutes for Cu at the ‘vacated’ histidine sites of Cu domains on PrP (15) and other cuproproteins such as the beta amyloid precursor protein. This could occur during periods of Cu deficiency,
when PrP subsequently loses its correct conformation, ‘metamorphosing’ into a misfolded PrP-like virus, Mn is released into the extracellular space so it can initiate lipid peroxidation and chain reactions of hydroxyl and Cu^3+ radical formation (10). Exposures to DEDTC and its parent compound sulfiram has been associated with the development of Parkinson’s disease and other extrapyramidal disorders (24–26), supporting the proposal that CNS Mn deficiency rather than CNS Cu overloading is associated with TSE aetiology.

**CU CHELATING CHEMICALS INDUCE Spongiform encephalopathies**

Cuprizone, neocuproine, mercaptoethanol, diethyldithiocarbamate and triethyltin are ‘true’ Cu chelating chemicals (20–22) which deplete Cu supplies in the CNS producing a ‘non-transmissible’, reversible type of spongiform encephalopathy. Cuprizone was actually utilised as a research tool for inducing a scrapie-like spongiosis in mice at Compton laboratories during the 1970s (23). CNS Cu deficiency could therefore represent one of the key prerequisites underlying TSE pathogenesis – albeit one which fails to account for the transmissible facet of TSEs.

**Is CNS Cu deficiency a prerequisite for TSEs?**

Certain facets of TSE pathogenesis, such as tissue increases of Fe stores, found as Ferritin (29), indicate a state of Cu/Fe deficiency.

Furthermore, Lung tissues of copper deficient chicks have demonstrated abnormal variations in the concentration of various types of glycoaminoglycans (30). The possibility of an ‘in vivo’ metabolic relationship between PrP and Cu deficiency could therefore represent one of the key prerequisites underlying TSE pathogenesis – albeit one which fails to account for the transmissible facet of TSEs.
and the glycoaminoglycans becomes evident after studying the TSE disease process (5); where concentrations of the sulphated glycoaminoglycans are raised in the CNS of diseased animals, whilst dramatic therapeutic benefits are witnessed ‘in vitro’ when TSE affected cell cultures are treated with these molecules. It seems likely that the normal cellular form of PrP (PrPc) binds with a specific co-species of glycoaminoglycans ‘in vivo’, which might serve as a means of protecting PrPc against conversion into its abnormal TSE isoform. This hypothesis proposes that CNS Cu deficiency is integral to the aetiology of TSEs, where the depleted supply of Cu to PrP’s Cu domain renders the protein vulnerable to invasion with alternative cations, such as Mn, which could ligate to the Cu domain and lead to the development of the misfolded, pathogenic prion associated with TSE.

CNS Cu deficiency can be invoked via various naturally occurring or artificially invoked mechanisms, or combinations thereof:

1. due to indigenous copper deficiency in the external food chain (influenced by seasonal, climatic and/or other geological (3,4,31) characteristics such as high soil molybdenum levels).
2. due to an oxidant mediated upregulation of the expression of Cu-metalloproteins, such as PrP, placing demands on the supply of available Cu in the CNS (32,15,33,34).
3. due to chelation of available Cu in the CNS by certain foreign organo pollutants (21).
4. due to the inhibitory effects placed on Cu absorption by excesses of Ca or estrogens (35,36) as in feeds such as alfalfa/soya respectively.
5. due to the accelerated excretion of Cu resulting from therapeutic treatment with steroids, etc (37).
6. due to a foreign organic pollutant induced covalent modification of (or binding to) the active histidine/tyrosine residues (10 p45) (12) (38) (39) on PrP’s Cu domain; thereby preventing Cu from accessing its binding domain on PrP (15,34).

Experimental evidence indicates a role for PrP’s Cu domain in protecting the cell against oxidative stress; via a PrP-mediated regulation of SOD1 activity.

D Brown and others have provided strong ‘in vitro’ and ‘in vivo’ experimental evidence that supports a functional role for PrPc in protecting CNS cerebellar cells against the deleterious impact of oxidative stress (33). Treatment with the antioxidant vitamin E has also been shown to protect cells lacking PrP expression against oxidant mediated cell death (33).

Brown proposes that PrPc influences the activity of SOD1 (40) and points to the Cu domain that has been identified at the N terminal octapeptide repeat region of PrP (15,34), suggesting that PrP may perform a role in the transportation of Cu to the sites of SOD 1 synthesis, where Cu and Zn act as co factors in the synthesis of the SOD 1 superoxide scavenger. Mice devoid of PrP due to genetic ablation demonstrate a reduced resistance to oxidative stress.

The key biochemical and pathological facets of TSE suggest a pivotal role of oxidative stress in TSE pathogenesis.

The biochemistry, pathology and distribution of CNS abnormalities associated with the pathogenesis of TSEs suggests that oxidative stress plays a major aetiological role in TSEs;

Decreased amounts of phospholipids and gangliosides/increased levels of cholesterol in membranes (41, p. 100), an abundance of lipofuscin inclusions in neurones (42), an upregulation of the signal transduction cycle and kinase C phosphorylation, increases in intraneuronal free calcium (43,44), reduction in monamine oxidase/NAD diaphorase (45,46), an increase in citrulline/ornithine in blood sera (47), a decrease in membrane fluidity (41, p. 106), rupturing of lysosomal membranes (46), an excessive CNS accumulation of iron in its Ferritin form (29), and a marked increase in multimeric mitochondrial DNA/DNA strand breakage (48). All of these abnormalities are characteristic of free radical disturbances (10,49–52) and are shared in the pathogenesis of other neurodegenerative diseases similar to TSEs such as Parkinsons (PD), Alzheimer’s (AD) and Motor Neurone Disease (MND) – diseases which are now recognised to stem from a free radical-mediated pathogenesis (52,53).

It is proposed that the different ‘strains’ of TSEs may be caused by different species of radical-generating divalent cation (Mn, Nickel, Fe or cobalt, etc) that can successfully compete and ligate at PrP’s Cu domain during states of Cu deficiency. The greater the oxidative capacity of the metal species involved (e.g. Mn 3+, Mn 4+ or even radioactive species), the more virulent strain of TSE to emerge. This aetiological model operates in combination with various other multifactorial criteria:

1. The duration and intensity of exposure to the different classes of divalent cation/organic chemical pollutant in the environment, (more directly related to the particular species of radical that they generate).
2. The TSE-susceptibility of the exposed individual; relating to their PrP (41)/SOD 1,2,3/ceruloplasmin/cytochrome P450 genotype.
3. Other external environmental variables which can ultimately influence the CNS uptake of Mn, e.g.: a. low Fe,
   b. levels of stress or environmental pollutants which mediate ACTH turnover, blood-brain barrier homeostasis and Mn uptake into CNS.
   c. daylight interval in relation to the ultra violet mediated regulation of melatonin turn over, which,
in turn, mediates estrogen/corticosteroid turn over and Mn uptake.

d. levels of estrogenic/steroid pollutants in the environment (4,54) and their ability to upregulate the expression of caeruloplasmin (55) which can lead to oxidation of increased amounts of Mn 2+ into its more lethal Mn 3+ species (7), particularly in the absence of its normal oxidation target, Fe 2+.

4. The developmental stage of the victim during intoxication (56).

**SPORADIC TSEs – AN ANALYTICAL SURVEY INTO THE LEVELS OF METALS IN ECOSYSTEMS SUPPORTING CLUSTERS OF TSE: DESIGN**

Foodchains supporting isolated, long standing clusters of sporadic TSEs were sampled by the author for analysis of mineral status in order to ascertain whether any elemental deficiencies or toxic excesses are common idiosyncratic characteristics of these TSE foci. Adjoining TSE-free regions populated by significant numbers of the respective species associated with the study were sampled as controls.

The TSE clusters of chronic wasting disease (CWD) in wild deer/elk in N. Central Colorado (57,58), sheep scrapie in N Iceland (59,60) and CJD in Slovakia (61–64) were selected for this survey for various reasons:

1. A long history of TSE being confined to specific well-defined regions.
2. The dependence of TSE affected populations on the local foodchain.
3. The relative freedom of those foodchains (excluding the Slovakia TSE foci) from excessive contamination by synthetic pro-oxidant industrial/agricultural pollutants which would Complicate the study.

The repeated failure of various Government TSE eradication programmes executed in the TSE endemic regions of Colorado/Iceland (57,59) – involving blanket slaughter, 4 year fallow, then restocking – suggests the persistent presence of a hitherto unrecognized environmental causal factor common to these regions.

It is worth noting that high altitude, snow covered peaked mountain ranges of volcanic origin – with an abundance of coniferous trees – characterize the environments where sporadic TSE foci have traditionally arisen. This could be linked to the annual spring snow thaw and the resulting waterlogging of soils; where the main minerals utilized as antioxidant cofactors in biological systems are leached out of the soil, whilst others, like manganese/aluminium, readily accumulate in the plant horizon as a result of the temporary acidic condition of the soils due to increases in anaerobic conditions during the wet season.

Furthermore, the hypoxia of high-altitude lining increases susceptibility to oxidative stress and increases permeability of the brain barrier to cations, etc.

**MATERIALS AND METHODS: SOIL**

Each soil sample comprised a representative sample drawn from a mix of approximately 20 slices of dry soil dug with a stainless steel trowel and taken at equal spacings along a W shape spanning an area of approximately five acres; the area being representative of the region grazed/cropped by the TSE affected mammals under study.

Each slice was drawn from the top soil to a depth of 6 inches, if possible, taking care to avoid inclusion of root material/surface organic matter and drawing of samples near gateways, roadsides, animal dung, disturbed/excavated or polluted terrain, etc.

The 20 slices were put into plastic bags and mixed together into an even homogenate, from which a further sample of no more than 300 g was drawn and placed into a small cardboard box which was sealed, labelled and dispatched to the UK Laboratories of Natural Resources Management (NRM), Coopers Bridge, Braziers Lane, Bracknell, Berkshire, RG42 6NS.

Soil samples were laid out on drying trays after arriving at the laboratory and dried in forced air flow cabinets for 12–18 h until dry. The temperature was maintained below 32°C for this period and the air was constantly dehumidified. Any solid rock granules were removed from samples before being ground to pass a 2 mm mesh using a hammer mill. The mill was flushed between samples using a small portion of the next sample. Samples required for total mineral analysis were subsequently ground to pass a 0.5 mm mesh. Samples were then presented for analysis – the analytical procedures for each mineral are described on Table 3, which were conducted in accord with the standard analysis procedure of the Ministry of Agriculture, Fisheries and Food.

Each plant tissue sample comprised a 200 g sample representing tissue collected from approximately 10 pickings taken at equal spacings in a W shape across an area of approx five acres which was representative of the region grazed/cropped by the TSE mammals under study.

Each picking involved taking tissue (leaves, stem and flowerheads) from the upper half of the plant, and involved species of grasses, plants and/or shrubs that comprised the overall dietary intake of the mammals under study according to local intelligence.

Samples were picked dry and away from roadsides, gateways, animal manure, polluted or disturbed terrain, whilst care was taken to avoid the inclusion of any root material or soil. The tissue was packed into plastic bags which were lightly sealed, labelled accordingly and dispatched to the laboratories of NRM.

Samples were thoroughly washed with deionised water, in a plastic sieve, on arrival at NRM Ltd. After removal of all roots and soil, the samples were spread evenly on a drying tray and dried in a 90 degree C oven.
to constant weight, and then ground by a Christy Norris mill. A small portion of the sample was used to flush the mill, before collection of the ground material. The samples were then prepared for analysis by dry ashing for non-volatile elements and wet digestion in aqua regia for volatile elements (e.g. selenium, etc).

The analytical procedures for each mineral is listed on Table 1, and these were carried out in accord with MAFF's standard analysis method.

**RESULTS: HIGH LEVELS OF MN CATION FOUND IN TSE ASSOCIATED FOODCHAIRS**

1. Icelandic scrapie cluster

The scrapie endemic valleys in North Central/Eastern Iceland where sheep have suffered from a high incidence of scrapie for many decades demonstrated a consistent two and a half fold greater concentration of the divalent cation, manganese, in herbage at 200 mg/kg dry basis in relation to the 80 mg/kg average level found in the regions where scrapie has never been recorded (Table 1) (Fig. 2).

This could be part related to the higher intensity of precipitation/snow cover (e.g., perhaps linked to the ecoimpact of the annual snow thaw run off) recorded in the scrapie endemic regions (personal communication; S. Sigurdarson) in combination with the high organic matter content of the peat soils which favours increased waterlogging and soil acidity, rendering Mn more freely available for plant uptake (31, p. 14/15/166).

Furthermore, the snow cover and short daylight interval of the Icelandic winters could favour an increased amount of Mn in herbage tissue in line with the recorded effects of shade on increasing the Mn content of leaves (65).

### Table 1

<table>
<thead>
<tr>
<th>Test Farm</th>
<th>N%</th>
<th>P%</th>
<th>k%</th>
<th>Mg</th>
<th>Ca%</th>
<th>Mn</th>
<th>Cu</th>
<th>Na%</th>
<th>Fe</th>
<th>Zn</th>
<th>Mo</th>
<th>Se</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scrapie-endemic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Efsti-Dalur</td>
<td>2.25</td>
<td>0.25</td>
<td>1.8</td>
<td>0.25</td>
<td>0.59</td>
<td>156</td>
<td>2.1</td>
<td>0.02</td>
<td>223</td>
<td>38.7</td>
<td>0.20</td>
<td>0.044</td>
<td>0.41</td>
</tr>
<tr>
<td>2 Vidivellir</td>
<td>1.56</td>
<td>0.20</td>
<td>1.26</td>
<td>0.19</td>
<td>0.57</td>
<td>89</td>
<td>2.4</td>
<td>0.05</td>
<td>118</td>
<td>32.7</td>
<td>2.68</td>
<td>0.018</td>
<td>0.15</td>
</tr>
<tr>
<td>3 Desjarmyri</td>
<td>2.35</td>
<td>0.28</td>
<td>1.40</td>
<td>0.24</td>
<td>0.39</td>
<td>228</td>
<td>3.4</td>
<td>0.32</td>
<td>599</td>
<td>47.6</td>
<td>0.56</td>
<td>0.032</td>
<td>0.40</td>
</tr>
<tr>
<td>4 Hrafnabjorg</td>
<td>3.15</td>
<td>0.36</td>
<td>1.63</td>
<td>0.24</td>
<td>0.44</td>
<td>107</td>
<td>5.0</td>
<td>0.06</td>
<td>164</td>
<td>27.8</td>
<td>0.41</td>
<td>0.012</td>
<td>0.28</td>
</tr>
<tr>
<td>5 Hofsa</td>
<td>2.64</td>
<td>0.30</td>
<td>1.33</td>
<td>0.18</td>
<td>0.41</td>
<td>144</td>
<td>4.7</td>
<td>0.12</td>
<td>389</td>
<td>34.0</td>
<td>0.73</td>
<td>0.055</td>
<td>0.32</td>
</tr>
<tr>
<td>6 Ingvarir (M)</td>
<td>1.88</td>
<td>0.21</td>
<td>1.17</td>
<td>0.17</td>
<td>0.40</td>
<td>297</td>
<td>3.5</td>
<td>0.05</td>
<td>942</td>
<td>32.9</td>
<td>1.07</td>
<td>0.051</td>
<td>0.59</td>
</tr>
<tr>
<td>7 Ingvarir (L)</td>
<td>2.85</td>
<td>0.29</td>
<td>0.90</td>
<td>0.31</td>
<td>0.90</td>
<td>145</td>
<td>4.3</td>
<td>0.35</td>
<td>132</td>
<td>17.5</td>
<td>3.29</td>
<td>0.029</td>
<td>1.61</td>
</tr>
<tr>
<td>8 Ingvarir (H)</td>
<td>1.06</td>
<td>0.10</td>
<td>0.81</td>
<td>0.12</td>
<td>0.26</td>
<td>277</td>
<td>1.2</td>
<td>0.01</td>
<td>151</td>
<td>18.2</td>
<td>0.48</td>
<td>0.010</td>
<td>0.25</td>
</tr>
<tr>
<td>9 pvera (M)</td>
<td>3.53</td>
<td>0.37</td>
<td>2.47</td>
<td>0.24</td>
<td>0.47</td>
<td>275</td>
<td>5.9</td>
<td>0.10</td>
<td>611</td>
<td>44.7</td>
<td>0.92</td>
<td>0.037</td>
<td>0.58</td>
</tr>
<tr>
<td>10 pvera (L)</td>
<td>1.62</td>
<td>0.17</td>
<td>1.05</td>
<td>0.20</td>
<td>0.60</td>
<td>245</td>
<td>2.3</td>
<td>0.01</td>
<td>213</td>
<td>31.6</td>
<td>0.62</td>
<td>0.110</td>
<td>0.42</td>
</tr>
<tr>
<td>11 pvera (H)</td>
<td>1.66</td>
<td>0.10</td>
<td>0.75</td>
<td>0.16</td>
<td>0.40</td>
<td>277</td>
<td>1.2</td>
<td>0.01</td>
<td>151</td>
<td>18.2</td>
<td>0.48</td>
<td>0.010</td>
<td>0.25</td>
</tr>
<tr>
<td>12 Atlastadir</td>
<td>1.58</td>
<td>0.20</td>
<td>0.94</td>
<td>0.21</td>
<td>0.46</td>
<td>310</td>
<td>3.0</td>
<td>0.03</td>
<td>192</td>
<td>20.7</td>
<td>0.17</td>
<td>0.011</td>
<td>0.13</td>
</tr>
<tr>
<td>13 Vígdisarstadir</td>
<td>3.30</td>
<td>0.32</td>
<td>0.58</td>
<td>0.34</td>
<td>0.51</td>
<td>210</td>
<td>6.5</td>
<td>0.24</td>
<td>271</td>
<td>28.3</td>
<td>1.26</td>
<td>0.010</td>
<td>0.46</td>
</tr>
<tr>
<td>Av scrapie</td>
<td>2.26</td>
<td>0.24</td>
<td>1.24</td>
<td>0.22</td>
<td>0.50</td>
<td>200</td>
<td>3.4</td>
<td>0.10</td>
<td>373</td>
<td>30.5</td>
<td>0.99</td>
<td>0.032</td>
<td>0.50</td>
</tr>
<tr>
<td>Category</td>
<td>mean low</td>
<td>mean low</td>
<td>mean low</td>
<td>very high</td>
<td>very low</td>
<td>mean low</td>
<td>mean low</td>
<td>very high</td>
<td>very low</td>
<td>mean low</td>
<td>mean low</td>
<td>very high</td>
<td></td>
</tr>
<tr>
<td><strong>Scrapie-free</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 Hjalp</td>
<td>1.73</td>
<td>0.21</td>
<td>1.18</td>
<td>0.29</td>
<td>0.84</td>
<td>89</td>
<td>2.3</td>
<td>0.03</td>
<td>303</td>
<td>34.4</td>
<td>0.51</td>
<td>0.077</td>
<td>0.40</td>
</tr>
<tr>
<td>15 Holmar</td>
<td>1.81</td>
<td>0.24</td>
<td>1.21</td>
<td>0.14</td>
<td>0.28</td>
<td>67</td>
<td>3.8</td>
<td>0.10</td>
<td>1285</td>
<td>24.2</td>
<td>2.20</td>
<td>0.021</td>
<td>0.76</td>
</tr>
<tr>
<td>16 Kvisker</td>
<td>2.10</td>
<td>0.25</td>
<td>1.62</td>
<td>0.38</td>
<td>0.77</td>
<td>100</td>
<td>3.2</td>
<td>0.08</td>
<td>98</td>
<td>122.3</td>
<td>0.86</td>
<td>0.030</td>
<td>0.16</td>
</tr>
<tr>
<td>17 Modruvellir</td>
<td>3.47</td>
<td>0.33</td>
<td>2.36</td>
<td>0.25</td>
<td>0.37</td>
<td>76</td>
<td>6.2</td>
<td>0.00</td>
<td>89</td>
<td>23.9</td>
<td>0.64</td>
<td>0.010</td>
<td>0.09</td>
</tr>
<tr>
<td>18 Modruvellir</td>
<td>2.52</td>
<td>0.28</td>
<td>2.34</td>
<td>0.17</td>
<td>0.31</td>
<td>69</td>
<td>6.2</td>
<td>0.01</td>
<td>61</td>
<td>14.2</td>
<td>0.38</td>
<td>0.010</td>
<td>0.23</td>
</tr>
<tr>
<td>19 Brakandi</td>
<td>1.90</td>
<td>0.18</td>
<td>1.71</td>
<td>0.17</td>
<td>0.33</td>
<td>96</td>
<td>2.1</td>
<td>0.00</td>
<td>85</td>
<td>16.4</td>
<td>1.26</td>
<td>0.020</td>
<td>0.14</td>
</tr>
<tr>
<td>20 Skriduklaustur</td>
<td>2.27</td>
<td>0.28</td>
<td>2.09</td>
<td>0.23</td>
<td>0.65</td>
<td>67</td>
<td>4.1</td>
<td>0.02</td>
<td>131</td>
<td>37.2</td>
<td>2.06</td>
<td>0.010</td>
<td>0.56</td>
</tr>
<tr>
<td>Av Sc-free</td>
<td>2.26</td>
<td>0.25</td>
<td>1.79</td>
<td>0.23</td>
<td>0.50</td>
<td>80</td>
<td>4.0</td>
<td>0.03</td>
<td>293</td>
<td>39.0</td>
<td>1.13</td>
<td>0.025</td>
<td>0.53</td>
</tr>
<tr>
<td>Category</td>
<td>mean low</td>
<td>mean low</td>
<td>mean low</td>
<td>mean low</td>
<td>mean low</td>
<td>very low</td>
<td>mean low</td>
<td>mean low</td>
<td>very high</td>
<td>mean low</td>
<td>mean low</td>
<td>very high</td>
<td></td>
</tr>
<tr>
<td><strong>Scrapie ?? in scrapie-endemic zone</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21 Sakka</td>
<td>3.41</td>
<td>0.35</td>
<td>1.88</td>
<td>0.23</td>
<td>0.48</td>
<td>179</td>
<td>6.1</td>
<td>0.11</td>
<td>417</td>
<td>48.5</td>
<td>1.88</td>
<td>0.020</td>
<td>0.43</td>
</tr>
<tr>
<td>22 Brautarholl</td>
<td>2.28</td>
<td>0.26</td>
<td>1.35</td>
<td>0.21</td>
<td>0.52</td>
<td>235</td>
<td>3.3</td>
<td>0.06</td>
<td>93</td>
<td>23.0</td>
<td>0.84</td>
<td>0.010</td>
<td>0.11</td>
</tr>
<tr>
<td>23 Barka</td>
<td>2.85</td>
<td>0.34</td>
<td>1.65</td>
<td>0.20</td>
<td>0.39</td>
<td>135</td>
<td>3.6</td>
<td>0.02</td>
<td>153</td>
<td>33.1</td>
<td>0.84</td>
<td>0.023</td>
<td>0.10</td>
</tr>
<tr>
<td>Av Sc ??</td>
<td>2.85</td>
<td>0.31</td>
<td>1.62</td>
<td>0.21</td>
<td>0.46</td>
<td>183</td>
<td>4.3</td>
<td>0.06</td>
<td>221</td>
<td>34.8</td>
<td>1.18</td>
<td>0.017</td>
<td>0.21</td>
</tr>
<tr>
<td>Category</td>
<td>mean low</td>
<td>mean low</td>
<td>mean low</td>
<td>mean low</td>
<td>mean low</td>
<td>very low</td>
<td>mean low</td>
<td>mean low</td>
<td>very high</td>
<td>mean low</td>
<td>mean low</td>
<td>very high</td>
<td></td>
</tr>
</tbody>
</table>

Levels of Al/S/V/Ni/Cr/F/As/Cd/Pb/Sn were normal on all farms tested.

Interestingly, there are some good examples of scrapie-free valleys found in the middle of the scrapie endemic zones which provide good opportunities for comparative studies. One fascinating example is demonstrated NW of Akureyri where the scrapie endemic valley ‘Svarfadrardalur’ runs 15 miles parallel to the scrapie free valley ‘Horgardalur’ (see Fig. 3). Sheep from both valleys freely intermingle on the open mountain during summer-time, suggesting that the mystery causal factor X associated with scrapie aetiology would be present in the specific valley homes where the scrapie affected flocks overwinter. Results of the author’s study demonstrated an av level of 94 mg/kg Mn (dry basis) drawn from 4 test sites in the scrapie free valley and 223.4 mg/kg Mn from 10 sites in the scrapie valley. Interestingly, Barka was the only farm recorded in the scrapie free valley that has purportedly suffered a suspected outbreak of scrapie in 1949, perhaps explaining why the Barka sample demonstrated the highest Mn level in the valley:

<table>
<thead>
<tr>
<th>SCRAPIE VALLEY</th>
<th>Mn mg/kg</th>
<th>SCRAPIE-FREE VALLEY</th>
<th>Mn mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ingvarir (M)</td>
<td>297</td>
<td>11 Modruvellir (H)</td>
<td>76</td>
</tr>
<tr>
<td>2 Ingvarir (L)</td>
<td>145</td>
<td>12 Modruvellir (L)</td>
<td>69</td>
</tr>
<tr>
<td>3 Ingvarir (H)</td>
<td>277</td>
<td>13 Brakandi</td>
<td>96</td>
</tr>
<tr>
<td>4 Pvera (M)</td>
<td>275</td>
<td>14 Barka</td>
<td>135</td>
</tr>
<tr>
<td>5 Pvera (L)</td>
<td>245</td>
<td>(scrapie rep 1930–1949)</td>
<td></td>
</tr>
<tr>
<td>6 Pvera (H)</td>
<td>127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Atlastadir</td>
<td>310</td>
<td>Av Mn dry basis</td>
<td>94</td>
</tr>
<tr>
<td>8 Sakka</td>
<td>179</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Brautarholl</td>
<td>235</td>
<td>(M) = mountainside sample.</td>
<td></td>
</tr>
<tr>
<td>10 Hofsa</td>
<td>144</td>
<td>(L) = lowland sample.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(H) = upland sample.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Av Mn dry basis</td>
<td>223.4</td>
</tr>
</tbody>
</table>

The recent fall in scrapie incidence in the scrapie-endemic regions must be partly due to the sharp decline in the total number of ‘TSE susceptible’ sheep due to the Icelandic government’s scrapie slaughter policies (60). The fall could also be due to the virtual universal switch over from feeding hay to silage as winter fodder over the last ten years in Iceland. Various analytical studies have demonstrated increasing concentrations of Mn in the seed heads of grasses during the maturation process (31), confirming the fact that manganese concentrations are higher in hay than in silage (69); simply because it is customary to harvest grass for hay at a more advanced stage of maturity than the younger flowering stage required for the silage harvest. Hidiroglou et al. (66) measured serum Mn levels in different batches of cattle fed hay or silage, and concluded that the bioavailability of Mn is much greater in hay than in silage.

2. Colorado CWD cluster (Tables 2 & 3)

Herbage drawn from an 80 mile cross section of the CWD endemic cluster zone in North central Colorado (Fig. 4) consistently demonstrated’ excessive levels of the divalent cation, calcium, at 1.19% total dry matter. However, the levels of Mn recorded in this specific batch of herbage/soil samples were low; averaging out at 39.5 mg/kg in herbage and 9.3 ppm in the soil. These samples were drawn during the drought conditions of July 1998 following three months of dry weather. (NB. Soil Mn is rendered considerably less available during drought conditions (31) whilst the protracted daylight of the mid summer period decreases levels of Mn in plant tissues.
Ecosystems supporting TSEs demonstrate excesses of the pro-oxidant manganese and Cu, Se, Fe, and Zn deficiencies (285).

However, soil sampling carried out the previous autumn on 13–25 October 1997 across the CWD cluster zone-following a period of rain and snow – recorded considerably higher levels of Mn averaging out at 317 mg/kg from the same test locations. It is possible that the recent increase in acid rainfall occurring along the CWD section of the Frong Range during the winter rain/snow season is also assisting an increased uptake of ‘available Mn’ from the soil into the herbage. Ca also averaged high concentrations of 2.55% dry matter in the autumn 1997 tests.

### Table 2
Analyses of herbage samples drawn across the CWD-endemic region of the Colorado Front Range on 12/7/98 – 16/7/98; readings in mg/kg dry basis, unless marked % w/w dry basis

<table>
<thead>
<tr>
<th>Location</th>
<th>N%</th>
<th>K%</th>
<th>Ca%</th>
<th>Cu</th>
<th>Fe</th>
<th>Mo</th>
<th>Se</th>
<th>P%</th>
<th>Mg%</th>
<th>Mn</th>
<th>Na%</th>
<th>Zn</th>
<th>B</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWD region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Horsetooth</td>
<td>1.66</td>
<td>1.89</td>
<td>0.77</td>
<td>5.8</td>
<td>84.8</td>
<td>0.10</td>
<td>0.08</td>
<td>0.15</td>
<td>0.20</td>
<td>38</td>
<td>0.01</td>
<td>19.0</td>
<td>19.8</td>
<td>0.90</td>
</tr>
<tr>
<td>5 Owl Canyon</td>
<td>2.63</td>
<td>1.46</td>
<td>2.04</td>
<td>9.1</td>
<td>80.7</td>
<td>2.03</td>
<td>0.25</td>
<td>0.21</td>
<td>0.40</td>
<td>30</td>
<td>0.01</td>
<td>19.2</td>
<td>36.6</td>
<td>1.60</td>
</tr>
<tr>
<td>6 Poudre Canyon</td>
<td>1.54</td>
<td>1.67</td>
<td>0.76</td>
<td>5.0</td>
<td>69.7</td>
<td>0.16</td>
<td>0.24</td>
<td>0.19</td>
<td>0.18</td>
<td>37</td>
<td>0.00</td>
<td>19.7</td>
<td>19.6</td>
<td>0.10</td>
</tr>
<tr>
<td>7 Teds Place</td>
<td>5.03</td>
<td>5.81</td>
<td>2.05</td>
<td>7.1</td>
<td>194.8</td>
<td>1.68</td>
<td>0.05</td>
<td>0.40</td>
<td>0.44</td>
<td>44</td>
<td>0.01</td>
<td>25.9</td>
<td>42.9</td>
<td>0.20</td>
</tr>
<tr>
<td>9 Black Canyon</td>
<td>2.21</td>
<td>1.83</td>
<td>0.71</td>
<td>6.6</td>
<td>365.7</td>
<td>1.06</td>
<td>0.64</td>
<td>0.26</td>
<td>0.16</td>
<td>50</td>
<td>0.00</td>
<td>105.7</td>
<td>18.2</td>
<td>0.04</td>
</tr>
<tr>
<td>12 H-Bar-G Ranch</td>
<td>1.76</td>
<td>1.92</td>
<td>0.83</td>
<td>5.6</td>
<td>131.9</td>
<td>1.16</td>
<td>0.27</td>
<td>0.24</td>
<td>0.19</td>
<td>38</td>
<td>0.00</td>
<td>45.0</td>
<td>21.9</td>
<td>0.50</td>
</tr>
</tbody>
</table>

### Table 3
Analyses of topsoils drawn across the CWD cluster zone of the North Central Colorado Front Range and a CWD-free zone in Utah on 12–16/7/98 and 20/9/97 respectively

<table>
<thead>
<tr>
<th>Test site</th>
<th>pH</th>
<th>P%</th>
<th>K%</th>
<th>Mg</th>
<th>Cu</th>
<th>B</th>
<th>Na</th>
<th>Zn</th>
<th>Ca</th>
<th>Mo</th>
<th>Fe</th>
<th>S</th>
<th>Se</th>
<th>Mn</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWD ZONE COLORADO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Horsetooth Mt</td>
<td>7.0</td>
<td>6.0</td>
<td>250</td>
<td>309</td>
<td>1.4</td>
<td>0.7</td>
<td>3.3</td>
<td>1.0</td>
<td>2576</td>
<td>0.0</td>
<td>14</td>
<td>36.7</td>
<td>.09</td>
<td>6.4</td>
<td>333</td>
</tr>
<tr>
<td>2 Spring Creek</td>
<td>6.6</td>
<td>3.8</td>
<td>115</td>
<td>167</td>
<td>1.3</td>
<td>0.4</td>
<td>2.4</td>
<td>0.9</td>
<td>1541</td>
<td>0.1</td>
<td>28</td>
<td>23.1</td>
<td>.18</td>
<td>15.0</td>
<td>261</td>
</tr>
<tr>
<td>3 Horsetooth Re</td>
<td>6.8</td>
<td>4.0</td>
<td>101</td>
<td>280</td>
<td>0.8</td>
<td>0.5</td>
<td>3.5</td>
<td>0.9</td>
<td>1635</td>
<td>0.1</td>
<td>22</td>
<td>27.1</td>
<td>.24</td>
<td>8.0</td>
<td>300</td>
</tr>
<tr>
<td>4 Livermore</td>
<td>8.7</td>
<td>5.4</td>
<td>290</td>
<td>72</td>
<td>1.0</td>
<td>0.8</td>
<td>1.4</td>
<td>1.1</td>
<td>2833</td>
<td>0.0</td>
<td>2</td>
<td>34.4</td>
<td>.09</td>
<td>2.0</td>
<td>373</td>
</tr>
<tr>
<td>5 Owl Canyon</td>
<td>8.6</td>
<td>6.0</td>
<td>221</td>
<td>153</td>
<td>0.9</td>
<td>0.7</td>
<td>5.2</td>
<td>0.9</td>
<td>3777</td>
<td>0.1</td>
<td>11</td>
<td>32.9</td>
<td>.19</td>
<td>5.0</td>
<td>229</td>
</tr>
<tr>
<td>6 Poudre Canyon</td>
<td>7.4</td>
<td>7.4</td>
<td>247</td>
<td>97</td>
<td>2.7</td>
<td>0.7</td>
<td>2.0</td>
<td>2.8</td>
<td>1512</td>
<td>0.0</td>
<td>16</td>
<td>24.1</td>
<td>.10</td>
<td>9.0</td>
<td>465</td>
</tr>
<tr>
<td>7 Teds Corner</td>
<td>6.8</td>
<td>25.4</td>
<td>506</td>
<td>99</td>
<td>2.5</td>
<td>0.8</td>
<td>3.9</td>
<td>3.0</td>
<td>2251</td>
<td>0.1</td>
<td>37</td>
<td>33.2</td>
<td>.19</td>
<td>18.0</td>
<td>320</td>
</tr>
<tr>
<td>8 Bellvue</td>
<td>8.3</td>
<td>29.2</td>
<td>236</td>
<td>248</td>
<td>2.1</td>
<td>1.0</td>
<td>9.9</td>
<td>1.7</td>
<td>3295</td>
<td>0.1</td>
<td>9</td>
<td>42.9</td>
<td>.13</td>
<td>3.0</td>
<td>99</td>
</tr>
<tr>
<td>9 Black Canyon</td>
<td>6.7</td>
<td>29.4</td>
<td>395</td>
<td>170</td>
<td>3.8</td>
<td>0.8</td>
<td>4.3</td>
<td>9.1</td>
<td>1418</td>
<td>0.3</td>
<td>50</td>
<td>21.9</td>
<td>.16</td>
<td>20.0</td>
<td>329</td>
</tr>
<tr>
<td>10 Black Canyon</td>
<td>6.4</td>
<td>5.6</td>
<td>175</td>
<td>146</td>
<td>0.9</td>
<td>0.5</td>
<td>3.1</td>
<td>1.5</td>
<td>1822</td>
<td>0.3</td>
<td>51</td>
<td>19.2</td>
<td>.16</td>
<td>10.0</td>
<td>468</td>
</tr>
<tr>
<td>11 H-Bar-G Ranch</td>
<td>6.6</td>
<td>6.6</td>
<td>249</td>
<td>150</td>
<td>1.3</td>
<td>0.5</td>
<td>1.9</td>
<td>0.8</td>
<td>1194</td>
<td>0.1</td>
<td>29</td>
<td>17.0</td>
<td>.06</td>
<td>7.0</td>
<td>341</td>
</tr>
<tr>
<td>12 H-Bar-G Ranch</td>
<td>6.5</td>
<td>4.8</td>
<td>167</td>
<td>110</td>
<td>1.4</td>
<td>0.5</td>
<td>3.9</td>
<td>2.9</td>
<td>1260</td>
<td>0.1</td>
<td>38</td>
<td>18.7</td>
<td>.12</td>
<td>8.0</td>
<td>293</td>
</tr>
</tbody>
</table>

### CWD-FREE UTAH

| Category          | 7.2 | 10.6| 246 | 166 | 1.6 | 0.6 | 3.7 | 2.2 | 2093| 0.1 | 25  | 27.6| .14 | 9.3 | 317|
| CWD-free          |     |     |     |     | very| low | very| low | very| low | very| very| very| high|     |

| Gt Cottonwood     | 7.6 | 11.4| 194 | 177 | 10.7| 0.6 | 275 | 61  | 2056| 0.2 | 52  | 67.7| .26 | 25.0|
| Lt Cottonwood     | 7.2 | 9.4 | 91  | 75  | 5.1 | ×   | ×   | 8   | ×   | 1.3 | 88  | ×   | 32.0|

### All samples analysed by National Resources Management Ltd on dry basis. × = insufficient sample for test. Cu/Zn were as EDTA extractable mg/l. Na/Ca/K/Mg were as ammonium nitrate extractable mg/l. Fe/Mn were as DPTA extractable mg/l. B as hot water soluble mg/l. Mo as Tannam extractable mg/l. S as available phosphate buffer soluble mg/l. Se as 'total' mg/kg. P as sodium bicarbonate extractable mg/l. Levels of Sn/Hg/F/I/Cr/Ni/Co/Pb/As/Al/V/Cd were normal at all locations tested.
the increase in population density of deer and elk in the ‘Rocky Mountain National Park’ region – the epicentre of the CWD cluster. They also report an increase in the consumption of pine needles by deer/elk in the overpopulated region, who have progressively switched onto this abnormal substitute ration since competition for the limited supplies of normal foods has increased. Pine needles contain high concentrations of certain cations, particularly in acid rainbelt districts; Mn being recorded at excessive concentrations ranging between 214–5810 p.p.m. (67).

3. Slovakia CJD cluster (Tables 4–7)

CJD has erupted in two distinct isolated foci in central Slovakia (61); one in the north in the Orava district where CJD cases have erupted in a remote group of neighbouring villages located along the western front range of the High Tatra mountains (Fig. 5). And then a smaller cluster of cases in the south, centred around the rural village of Poltar (62–64).

Eva Mitrova has identified a genetic risk factor associated with the Slovak CJD foci, but also points to the presence of some hitherto unidentified environmental factor that plays a crucial role in the aetiology of CJD in these two high-risk foci (61).

Despite a protracted spell of sunny, dry weather (which prevents Mn accumulation in plants (31,65)) prior to the sampling period in September 1999, Mn levels were generally high in the CJD region. A 2 1/2 fold higher concentration of Mn was recorded in the vegetation of the uncultivated pastures of the Orava CJD cluster region in relation to a control sample drawn across a CJD-free area 100 miles East near Poprad (Table 4). Extractable Mn Levels were ‘excessive’ at 437 mg/kg in Zuberec – the epicentre of the Northern CJD foci (61).

Sampling was largely concentrated around the village of Zuberec in the Orava CJD cluster region (see Fig. 5) and the village of Poltar in the Southern CJD foci because of the high 1 in 1000 CJD risk attached to the residents of these villages (61). Sampling was also carried out in Pucov, since scrapie was first isolated in the sheep of this region (68), although scrapie was thought to have existed more extensively in sheep residing throughout the whole Orava region. Interestingly, pastures in Pucov demonstrated a
Table 4 Analyses of herbage/foods sampled across the Slovak CJD cluster zones of Orava/Poltar and CJD-free region of Poprad 23/9/99 to 30/9/99; in mg/kg dry basis, unless marked % w/w

<table>
<thead>
<tr>
<th>Test site</th>
<th>P%</th>
<th>K%</th>
<th>Mg%</th>
<th>Ca%</th>
<th>Mn</th>
<th>Cu</th>
<th>Na%</th>
<th>Fe</th>
<th>Zn</th>
<th>Mo</th>
<th>Se</th>
<th>Al</th>
<th>Co</th>
<th>S%</th>
<th>Ni</th>
<th>Ti</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Orava cluster)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zuberec</td>
<td>.12</td>
<td>1.01</td>
<td>.23</td>
<td>0.94</td>
<td>437</td>
<td>5.9</td>
<td>.01</td>
<td>83.5</td>
<td>74.2</td>
<td>3.0</td>
<td>.052</td>
<td>84.4</td>
<td>.34</td>
<td>.20</td>
<td>4.35</td>
<td>1.35</td>
</tr>
<tr>
<td>Huty</td>
<td>.19</td>
<td>1.82</td>
<td>.26</td>
<td>1.85</td>
<td>86</td>
<td>8.1</td>
<td>.01</td>
<td>87.1</td>
<td>40.9</td>
<td>3.8</td>
<td>.043</td>
<td>108.5</td>
<td>.17</td>
<td>.32</td>
<td>1.50</td>
<td>0.30</td>
</tr>
<tr>
<td>Malatina</td>
<td>.28</td>
<td>2.20</td>
<td>.28</td>
<td>1.58</td>
<td>115</td>
<td>9.9</td>
<td>.01</td>
<td>119.6</td>
<td>36.6</td>
<td>2.6</td>
<td>.043</td>
<td>115.2</td>
<td>.19</td>
<td>.25</td>
<td>5.41</td>
<td>1.41</td>
</tr>
<tr>
<td>Pucov</td>
<td>.20</td>
<td>1.98</td>
<td>.27</td>
<td>1.99</td>
<td>204</td>
<td>6.9</td>
<td>.01</td>
<td>111.6</td>
<td>33.2</td>
<td>0.8</td>
<td>.041</td>
<td>102.6</td>
<td>.23</td>
<td>.19</td>
<td>10.8</td>
<td>1.19</td>
</tr>
<tr>
<td>Av CJD</td>
<td>.19</td>
<td>1.75</td>
<td>.26</td>
<td>1.59</td>
<td></td>
<td>210</td>
<td>7.7</td>
<td>.01</td>
<td>100.4</td>
<td>46.2</td>
<td>2.5</td>
<td>.044</td>
<td>102.7</td>
<td>.23</td>
<td>.24</td>
<td>5.51</td>
</tr>
<tr>
<td>Scale</td>
<td>low</td>
<td>norm</td>
<td>low</td>
<td>very high</td>
<td></td>
<td>high</td>
<td>low</td>
<td>low</td>
<td>low</td>
<td>norm</td>
<td>very</td>
<td>low</td>
<td>?</td>
<td>high</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>CJD-free (Poprad)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poprad S</td>
<td>.41</td>
<td>2.64</td>
<td>.34</td>
<td>1.57</td>
<td></td>
<td>85</td>
<td>15</td>
<td>.02</td>
<td>166.0</td>
<td>34.2</td>
<td>0.6</td>
<td>.032</td>
<td>182.4</td>
<td>.31</td>
<td>.37</td>
<td>23.3</td>
</tr>
<tr>
<td>Scale</td>
<td>high</td>
<td>norm</td>
<td>norm</td>
<td>very high</td>
<td></td>
<td>norm</td>
<td>high</td>
<td>very</td>
<td>low</td>
<td>norm</td>
<td>low</td>
<td>norm</td>
<td>very</td>
<td>low</td>
<td>?</td>
<td>high</td>
</tr>
</tbody>
</table>
| Matrix 2 – Pine needles

| CJD Endemic (Orava cluster) |      |       |       |      |      |      |     |     |      |     |     |      |     |    |     |      |
| Zuberec                   |      | 951   | 3.9   |      | 104  | 52.3 |      |     |      |     | 103.0|     | 33.5| 1.23|
| Scale                      | very high | very low | low | norm |      |      |     |     |     |     |      |     |     |     |     |
| CJD-free (Poprad)          |      |       |       |      |      |      |     |     |      |     |     |      |     |    |     |      |
| Vernar                     |      | 59    | 3.2   |      | 113  | 57.1 |      |     |      |     | 76.7 |     | 19.2| 1.98|
| Scale                      | mean | very low | low | norm |      |      |     |     |     |     |      |     |     |     |     |

Table 5 Analyses of specific crops cultivated on allotments within the Orava/Poltar CJD endemic regions 23/9/99 to 30/9/99; in Mg/Kg dry basis or % w/w dry basis

<table>
<thead>
<tr>
<th>Alfalfa (Zuberec)</th>
<th>Alfalfa (Poltar)</th>
<th>Alfalfa (Parnica)</th>
<th>Nuts (Zuberec)</th>
<th>Potatoes (Zuberec)</th>
<th>Cabbage (Poltar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P%</td>
<td>0.35 (.4)</td>
<td>0.29</td>
<td>0.30</td>
<td>0.26 (.04)</td>
<td>0.32</td>
</tr>
<tr>
<td>k%</td>
<td>2.59</td>
<td>2.21</td>
<td>2.30</td>
<td>2.36</td>
<td>2.70</td>
</tr>
<tr>
<td>Mg%</td>
<td>0.25 (.54)</td>
<td>0.20</td>
<td>0.27</td>
<td>0.17 (.03)</td>
<td>0.25</td>
</tr>
<tr>
<td>Ca%</td>
<td>2.46 (2.1)</td>
<td>1.64</td>
<td>1.53</td>
<td>1.42 (.20)</td>
<td>1.36</td>
</tr>
<tr>
<td>Mn</td>
<td>53 (37)</td>
<td>70.0</td>
<td>38.00</td>
<td>149.0 (35)</td>
<td>9.00 (7.0)</td>
</tr>
<tr>
<td>Cu</td>
<td>9.8 (9.1)</td>
<td>6.9</td>
<td>9.60</td>
<td>6.2 (14)</td>
<td>3.20</td>
</tr>
<tr>
<td>Na%</td>
<td>0.02 (.07)</td>
<td>0.01</td>
<td>0.01</td>
<td>.00 (.01)</td>
<td>0.02</td>
</tr>
<tr>
<td>Fe</td>
<td>120.2 (291)</td>
<td>99.7</td>
<td>104.10</td>
<td>98.5</td>
<td>70.20 (26)</td>
</tr>
<tr>
<td>Zn</td>
<td>33.6</td>
<td>31.1</td>
<td>36.80</td>
<td>10.3 (34)</td>
<td>18.70</td>
</tr>
<tr>
<td>Al</td>
<td>97.2</td>
<td>73.5</td>
<td>78.3</td>
<td>22.3</td>
<td>98.50</td>
</tr>
<tr>
<td>Mo</td>
<td>3.20</td>
<td>0.60</td>
<td>90.60</td>
<td>5.40</td>
<td>5.40</td>
</tr>
<tr>
<td>Se</td>
<td>0.033</td>
<td>0.03</td>
<td>0.04</td>
<td>0.024</td>
<td>0.033</td>
</tr>
<tr>
<td>Co</td>
<td>0.160 (.15)</td>
<td>0.03</td>
<td>0.17</td>
<td>0.30</td>
<td>0.43</td>
</tr>
<tr>
<td>S%</td>
<td>0.36</td>
<td>0.27</td>
<td>0.23</td>
<td>0.42</td>
<td>0.63</td>
</tr>
<tr>
<td>Ni</td>
<td>3.49</td>
<td>45.90</td>
<td>13.30</td>
<td>46.4</td>
<td>55.80</td>
</tr>
<tr>
<td>Ti</td>
<td>0.90</td>
<td>1.47</td>
<td>0.95</td>
<td>0.55</td>
<td>2.16</td>
</tr>
</tbody>
</table>

Bracketed figure indicates standard levels of element normally recorded in that specific crop. Source refs: (69) (4) (3).
similar Mn/Cu status (Table 4) to that recorded in Icelandic scrapie endemic regions.

As residents of the Orava region have largely lived a self-sufficient lifestyle growing their foods on allotments surrounding the villages (61), samples of some of their mainstay foods (e.g. potatoes, nuts and cabbage) were taken for analysis. Results demonstrated levels of Mn in excess of the average Mn levels usually associated with these crops (Table 5).

Mn was deficient in samples of the tap and river water supplies currently supplying Zuberec village. However, significant amounts of Mn were detected in the tap water of nearby Malatina village within the CJD region (63) and in Poltar village in the Southern CJD cluster region. Mn was absent in the watersupplies of the CJD-free region (Table 6).

<table>
<thead>
<tr>
<th>Location</th>
<th>source</th>
<th>Se mg/l</th>
<th>Mg mg/l</th>
<th>Ca mg/l</th>
<th>Al mg/l</th>
<th>Cu mg/l</th>
<th>Fe mg/l</th>
<th>Mn mg/l</th>
<th>Co mg/l</th>
<th>Ni mg/l</th>
<th>Cr mg/l</th>
<th>S mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poltar</td>
<td>tap</td>
<td>&lt;3</td>
<td>3.58</td>
<td>21.1</td>
<td>&lt;1</td>
<td>2.76</td>
<td>95.6</td>
<td>31.4</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>18.9</td>
</tr>
<tr>
<td>Poltar</td>
<td>river</td>
<td>&lt;3</td>
<td>3.93</td>
<td>23.5</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1.5</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>18.1</td>
</tr>
<tr>
<td>Poltar</td>
<td>tap</td>
<td>&lt;3</td>
<td>2.64</td>
<td>27.2</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>39.9</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>30.6</td>
</tr>
<tr>
<td>Malatina</td>
<td>tap</td>
<td>6.3</td>
<td>24.30</td>
<td>135.9</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>34.4</td>
<td>2.3</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>39.7</td>
</tr>
<tr>
<td>Zuberec</td>
<td>tap</td>
<td>&lt;3</td>
<td>4.86</td>
<td>22.7</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>Zuberec</td>
<td>river</td>
<td>&lt;3</td>
<td>3.35</td>
<td>17.9</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>mean CJD</td>
<td></td>
<td>2.3</td>
<td>7.11</td>
<td>41.4</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>8.7</td>
<td>23.0</td>
<td>11.4</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>21.1</td>
</tr>
<tr>
<td>CJD-FREE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poprad</td>
<td>tap</td>
<td>8.7</td>
<td>57.50</td>
<td>217.7</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>3.64</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>321.4</td>
<td></td>
</tr>
</tbody>
</table>

As residents of the Orava region have largely lived a self-sufficient lifestyle growing their foods on allotments surrounding the villages (61), samples of some of their mainstay foods (e.g. potatoes, nuts and cabbage) were taken for analysis. Results demonstrated levels of Mn in excess of the average Mn levels usually associated with these crops (Table 5).

Mn was deficient in samples of the tap and river water supplies currently supplying Zuberec village. However, significant amounts of Mn were detected in the tap water of nearby Malatina village within the CJD region (63) and in Poltar village in the Southern CJD cluster region. Mn was absent in the watersupplies of the CJD-free region (Table 6).

With one exception, Cu and Se were undetectable in all water supplies sampled in the CJD cluster regions. A similar analytical survey (70) was conducted at the Liptovsky Mikulas Health Institute in 1998, where water supplies in the Lipt Sielnica vicinity of the Northern CJD foci were all found to be markedly deficient in Mn/Cu/Se/Zn/Fe.
Ecosystems supporting TSEs demonstrate excesses of the pro-oxidant manganese and Cu, Se, Fe, and Zn deficiencies.

Considering the therapeutic effects of the sulphate ion in arresting the TSE disease process in scrapie affected cell cultures (5), the sixteen fold raised level of sulphate in the CJD-free water supplies in relation to CJD endemic supplies is interesting.

The concentration of metals in Pine needles serves as a sound yardstick for assessing the levels of metal contamination of ecosystems (67); particularly relevant when assessing the levels of atmospheric metal particulates.

Interestingly, samples of needles collected across a five acre stand of pine trees around CJD endemic Zuberec yielded 951 mg/kg of Mn, whilst a similar collection of needles sampled across the CJD-free region of Poprad yielded only 59 mg/kg of Mn.

Investigation of the environments around the CJD foci for potentially unique sources of atmospheric Mn contamination identified the presence of two large ferromanganese factories sited in the Orava valley at Siroka and Istebne along the North Western boundary of the Northern CJD focus, and the presence of a glass factory (64) (Mn is employed in the glass making process (4)) in Poltar in the Southern CJD foci. Some of the CJD victims had been employed in these factories for varying periods of their working lives. So people working at or living downwind of these factories would have been exposed to significant levels of airborne manganese and silicates in both the Northern and Southern CJD foci.

The factories were originally constructed during the communist era at a time when scant resources were channelled into curtailing chimney emissions of toxic pollutants. Emissions from these factories are locally renowned to form clouds of 'Smog' which travel up the valleys in a Southerly/Easterly direction for several kilometres – precisely over the communities where CJD has erupted. Public fears of atmospheric pollution with manganese dioxide, nickel and other metal compounds downwind of these factories, prompted a study by the Dolny Kubin Health Institute (71) where the hair of children were analysed for metals in the towns of Dolny Kubin (in the Orava CJD cluster region) and Oravska Lesna (outside of the CJD endemic region).

Fig. 5 Distribution of CJD cases in the Northern Orava Valley cluster region of Slovakia in relation to location of ferromanganese factories. Source (63). 1949 + 1990 (example) = lifetime of CJD case written beside village of origin. M = ferromanganese factory. Malatina = village vicinity where samples were taken.
Interestingly, the results of the 1995 analyses (71) demonstrated 12.945 mg/kg Mn in children residing in the CJD-endemic Dolny Kubin and 2.832 mg/kg Mn in children residing in the CJD-free Oravska Lesna. Analyses of the other metals in children residing in the two regions did not demonstrate the same significant variation observed with Mn (see Fig. 6).

Fig. 7 demonstrates the decline of Mn levels in children of Dolny Kubin from 15.957 mg/kg Mn measured in 1983 to 9.500 mg/kg measured in 1987 with a subsequent rise back to 12.945 mg/kg measured in 1995. World Health Organization max limit for Mn is 4 mg/kg.

The map of CJD distribution in the Orava region (Fig. 5) demonstrates a North/South Easterly distribution of CJD in relation to the location of the ferromanganese factories along the Orava valley. The distribution of CJD in the villages amidst the Western foothills of the High Tatras correlates with a hypothetical scenario wherein the prevailing westerly winds pick up airborne metal particulates emitted from the factories and carry them the five to fifteen mile journey to the ‘rain belt’ foothill region, where rainfall delivers the metal pollutants back to the terrestrial ecosystem, contaminating any TSE susceptible genotypes (amongst the local sheep and human populations) dependent on local foodchains rendering them at high risk of developing TSE. The problem of atmospheric Mn contamination may have been compounded further by the presence of other ferromanganese plants located several kilometres away to the North across the Polish border.

Whilst a background incidence of CJD is thought to have existed in TSE susceptible genotypes in the Orava region for many decades (63), it is interesting that incidence rates of CJD did not start to rise until the 1950s (and later scrapie), then peaking later at the high rates encountered in the 1980s – perhaps reflecting a delayed neurotoxic response to the development of the ferromanganese industry in Orava (and glass production in Poltar), where vulnerable early life exposure of Cu deficient individuals to an Mn contaminated environment lead to the
formation of Mn misfolded prion protein in the CNS with clinical TSE manifesting many years later in adulthood.

**Deficient levels of radical scavenger cofactor metals Cu, Zn, Fe, Se in foodchains supporting all three TSE clusters sampled.**

The soils and herbage samples drawn from the CWD, scrapie and CJD cluster zones in Colorado, Iceland and Slovakia demonstrated marked deficiencies of Cu, Zn, Se, Fe, Na, Mg and P in common (Tables 1–3). For instance, the concentration of these elements in the soils of the Colorado CWD endemic zone were 5, 15, 1.8, 2.8 and 74 fold less respectively than their equivalent levels in samples taken from CWD-free areas in Utah State.

Interestingly, the Pronghorn antelope is the only species of free ranging ruminant in the Colorado TSE zone that has failed to contract CWD, despite their close cohabitation with CWD affected deer and elk (57, 58). Work by Clemens et al. (72) may offer an explanation for the pronghorn's CWD-free status. They demonstrated the pronghorn's unique ability to conserve/ regulate Se in Se deficient environments in relation to the markedly less efficient ability displayed by deer and bison. As Pronghorn are unique to the N American prairies – having evolved there over the last 20 million years (73) – they have obviously adapted to the challenges posed by their indigenous terrain and are likewise better equipped at maintaining adequate activities of the Se-activated glutathione peroxidase enzyme/ antioxidant vitamin E which may play preventative roles in scavenging radical cascades triggered off in those ruminants who are resident in ‘TSE endemic’ ecosystems.

Likewise, the plant material drawn from the Icelandic scrapie endemic regions also shared the same deficiencies of Cu, Se, Zn, Fe, Na, P, Mg found in Colorado/ Slovakia. But unlike Colorado/Slovakia, these elements were also deficient in the scrapie free valleys, suggesting the potential vulnerability of the entire Icelandic sheep population to scrapie, should levels of Mn suddenly rise for any reason – e.g via Mn contamination from a fresh outfall of volcanic ash (74) – whereby engaging the dual ‘toxic template’ theoretically required for triggering off TSE.

The results of the soil samples drawn from the same farms in Iceland during August/September 1998 demonstrated uniform levels of all elements on both the scrapie-free and scrapie endemic farms. Interestingly, the levels of most elements recorded in the soil were at the opposing end of the scale in relation to their levels in herbage; For instance, Mn levels were very low, Zn/Fe were high, Cu/molybdenum were excessive, whilst selenium maintained the same deficient status found in herbage.

The low levels of Cu in herbage is perhaps explained by the well recognised chelating action of Molybdenum on Cu. Thus explaining how the excess of Cu found in the soil is reduced to a state of ‘secondary Cu deficiency’ by the time it reaches the plant horizon, as a result of the chelating action exerted by the high levels of molybdenum in the soil.

The significant 21/2+ fold higher levels of Mn recorded in the herbage of the scrapie farms points to the presence of some critical environmental factor that increases uptake of Mn into herbage on the scrapie farms whilst remaining absent on scrapie-free farms.

**CONCLUSIONS DRAWN FROM TEST RESULTS**

These results add support to the hypothesis that TSE susceptible genotypes will succumb to sporadic TSE if they have been dependent on a ecosystem which demonstrates the following coexisting abnormalities in its mineral status:

1. Excessive levels of the divalent cation metal, manganese – which can act as a pro oxidant in Mn SOD deficient genotypes.
2. Deficient levels of Cu, Zn, Se and Fe; being critical cofactor components of the major groups of radical scavengers – the SODs catalases, glutathionine peroxidases, vitamin E (10).
3. Deficient levels of Cu, Fe, P and Mg; where low Cu/Fe/P/Mg induces an excessive absorption of divalent cation Mn (3, 4) and its ultimate accumulation in the CNS, and low Mg levels assist Mn 2+ to compete and substitute at various ‘Mg specific’ catalytic sites normally occupied by Mg2+ (3, 4, 7), leading to the failure of activation of enzymes like Mg activated ATP in CNS synaptosomes (75).

Mn absorption is accelerated during conditions of sub clinical Fe and Cu deficiency (3, 4), as well as in states of P deficiency (4, 31). Results of this survey suggest that this precise scenario is actualized ‘in vivo’ in mammals residing in these TSE ecosystems. The mineral deficiencies were recorded in herbage that was harvested during July/August, at the stage of the seasonal cycle when these elements have reached their peak concentrations (8). (NB, concentrations of Cu/Fe in herbage can oscillate by as much as 30 times around one seasonal cycle (8). This suggests that mineral levels would have measured lower if sampling had been carried out at any other time of the year, rendering susceptible mammals at a peak of vulnerability to TSEs in the winter/early spring period; especially relevant to those cervidae/ sheep residing in the protracted snowbound districts of Colorado’s Front Range / the High Tatras / the N Icelandic mountains who have only had access to ‘hay’ fodder – hay carrying lower concentrations of copper and higher concentrations of Mn than other types of winter feed (69, 66).
Whilst low Cu levels in Icelandic herbage is probably linked to the chelating action of the high molybdenum levels analysed in the soils, the low levels of Cu in the herbage of the Colorado and Slovakia TSE clusters is further compounded by the excessive levels of Ca analysed in the plant material drawn from both the CWD and CJD regions of Colorado and Slovakia. High levels of Ca in the diet would further exacerbate the already deficient levels of Cu in these regions by impairing Cu absorption in the gut of cervidae/humans due to Ca-mediated pH alterations (35). Furthermore, the prominent cultivation of the ‘high calcium’ alfalfa crop (69) in both of these cluster regions (Colorado alfalfa measured 2.05% Ca, Zuberec alfalfa 2.46% Ca) would further compound the problems of High Ca in the local foodchains. Interestingly, the captive deer that contracted CWD were fed on an almost exclusive diet of alfalfa (57) whilst the zoo animals associated with TSE outbreak had also been fed rations containing alfalfa.

**REVIEW OF THE MN LITERATURE IN RELATION TO THE HYPOTHETICAL PERSPECTIVE THAT MN 3+ SERVES AS THE ‘INFECTIOUS’ TRANSMISSIBLE AUTO-OXIDATIVE AGENT IN TSEs**

**Interspecies / interdevelopmental variations in the rate of Mn absorption, and its relationship to susceptibility to Mn intoxication.**

Interestingly, Mn is largely absorbed via the duodenum (3, 4), explaining the more efficient absorption rate of 10–18% of available dietary Mn in adult ruminants (such as cattle, sheep, goats and cervidae) in relation to the less efficient absorption rate of 2–5% of available Mn in the diet of monogastric species (such as pigs and poultry) (3, 4). The significant difference between the efficiency of Mn absorption in ruminants and monogastric species may partly explain why ruminants are prone to TSEs and monogastrics remain virtually TSE free (76, 41). However, airborn Mn is readily absorbed into the brain via the intranasal-olfactory route which may be relevant to some TSEs (9).

Mn absorption and retention is considerably increased in the fetus and infant, due to the immaturity of the duodenal/intestinal barriers and the immaturity of Mn's excretory pathways, such as the pancreatic juices and bile. Adult rats absorb 3–4% of orally administered Mn whilst young rats absorb 20% (4, p. 191). Hatano et al. (77) has reported erythrocyte Mn levels at 100 mg/1 in Japanese infants under 1 month old, whilst 35 mg/1 levels were recorded in Japanese adults. Miller et al. (78) has shown that manganese excretion is virtually negligible in the neonatal stages due to low bile output. Mena et al 1978 (3, p. 262) demonstrated that premature children have a 25 fold increase in Mn retention in relation to adults – according to the levels monitored fifty days after Mn ingestion.

Furthermore, trials (79) have indicated that there is a fourfold increased rate of entry of Mn into the brain of newborn rats in relation to adult rats – probably indicating the immaturity of the blood/brain barrier at these early stages. Another study demonstrated that the brain (80) of human stillborns contained an average of 46 ppm Mn, whereas brain tissue sampled from all of the post natal age groups (1 day to 80 yrs) consistently averaged 20 ppm Mn. It has also been demonstrated that there is a 100% increase in the rate of Mn's plasma binding and entry into the brain in Fe deficient rats (79) – demonstrating the possible relevance of the low Fe readings in the TSE ecosystems (Tables 1 & 2).

Mn will therefore accumulate more readily in early life due to the immaturity of the homeostatic mechanisms presiding over absorption and excretion, placing the embryo and infant in the highest risk category for susceptibility to Mn intoxication.

Mn absorption therefore decreases with age, suggesting that exposures to pathogenic levels of Mn during the vulnerable early life period could lead to a more virulent, early onset ‘strain’ of the Mn delayed neuropsychiatric syndrome. It is proposed that the BSE/ nv CJD ‘strain’ of TSE, which erupts at a relatively younger age than conventional sporadic TSEs (81), is the result of a significant in utero exposure to a more potent oxidative species of Mn (Mn 4+, Mn5+ or a radioactive Mn) in combination with exposure to Cu chelating insecticides, whereas sporadic TSEs are the result of post natal exposures to a less reactive oxidative species of Mn (Mn2+, Mn3+) in combination with a coexisting CNS Cu deficiency.

**The biochemistry, pathology and symptomology of Mn delayed psychoneurotoxicity exhibits strong similarities to that observed in TSEs.**

Mn largely concentrates in the pineal, pituitary, median eminence of the hypothalamus, basal ganglia and olfactory bulb of the brain, being found specifically in the melanocytes and in the mitochondria of astrocyte cells belonging to those regions, where it performs a major role in oxidation and reduction reactions (3, 4, 7).

Interestingly, exposures to toxic levels of divalent Mn manifests its pathogenicity selectively within the brain. However, when exposure to Mn involves the inhalation route, the lungs are also affected. CNS mitochondria do not possess a mechanism for clearance of Mn following contexts of overloading (7, 82).

Mn has been found in its Mn2+, Mn3+, Mn4+ valency states in living tissues, the higher valences being more highly reactive (7). Mn3+ complexes with transferrin and readily crosses membranes, and has a slower rate of elimination from tissues than Mn 2+ (7). Mn3+ also possesses
different affinities for endogenous ligands than the Mn 2+ species (7), thereby creating a different spectra of toxicological activity than that encountered during Mn 2+ overload.

There is evidence to suggest that divalent Mn is oxidized to trivalent Mn by ceruloplasmin in hepatocytes (83), and it should be noted here that exposure to estrogenic/steroid pollutants (55) (84), psychological stress (83) or neuropathic types of organo phosphates (OPs) (85), considerably upregulates the expression of ceruloplasmin, thus, presumably, accelerating the oxidative transformation of Mn 2+ in the liver into its more lethal pro-oxidative Mn 3+ species – particularly in contexts of Fe deficiency encountered in TSE ecosystems. (A greater part of ceruloplasmin’s normal activity involves the oxidation of its Fe2+ target (10)).

‘In vitro’ studies show that Mn 3+ complexes will readily auto-oxidise catecholamines, explaining why exposure of humans or animals to large doses of Mn considerably decreases the levels of dopamine, serotonin and noradrenaline in the striatum – particularly in the caudate nucleus and the putamen (10, 11, 86, 87). However, the dopamine-rich substantia nigra usually remains unaffected (11) in Mn intoxications.

Interestingly, the brains of scrapie affected sheep (88, 89, 92) / CJD affected humans (90, 91) have demonstrated the same pattern of dopamine, serotonin and noradrenaline depletion in the CNS as witnessed in Mn toxicity – where the putamen and caudate nuclei of the striatum is most intensively affected, whilst dopamine in the substantia nigra/mesolimbic system is largely preserved (88).

Various drug therapies such as bromocriptine, leperotri, phenothiazine and chlorpromazine (11, 93) considerably raise the levels of Mn in select areas of the brain producing extrapyramidal symptoms such as tardive dyskinesia in treated subjects, thus elucidating the interactive role which Mn performs with dopamine in these disorders. Furthermore, treatment of those suffering from the early stages of Mn neuropsychiatric syndrome with the dopamine-precursor drug L-DOPA (administered in PD) causes a dramatic ephemeral remission of major symptoms (3, 11, 94).

Raised levels of GABA have also been recorded in the caudate nucleus of rats exposed to toxic levels of Mn (11, 95). A rise in GABA has similarly been recorded in TSEs, which is presumed to stem from a loss of synaptic inhibition at the GABA receptors (96).

The pathological perspective of Mn intoxication

The pathology of Mn poisoning varies according to the genetic idiosyncrasies of the victim, the valency of the specific Mn species involved and the specific Mn-protein conjugate involved (7), etc. However, all cases generally demonstrate a common pathological hallmark involving shrinkage and distortion of the basal ganglia with the destruction of its ganglion cells, particularly in the caudate nuclei and the putamen (97). These and other pathological features of Mn intoxication such as astrogliosis / amyloid plaques composed of bundles of fibrils /neuronal loss / atrophy in many CNS regions (6) are duplicated in the victims of CJD, kuru, scrapie and other TSEs (41).

The ventricles are enlarged in sections of CJD brain (41) – a phenomena that has not been reported in the limited number of pathological investigations into Mn intoxications carried out during the first half of this century. However, the ventricles were moderately enlarged in a CT and MRI scan of a recent case of poisoning with the Mn fungicide, Maneb (28). EEGs show a diffuse slowing following some Mn intoxications (98, 99) – a pattern usually recorded in sporadic TSEs (41).

Other regions lesioned in chronic Mn intoxications are the globus pallidus in the basal ganglia, as well as the pyramidal system, the cerebellum and its connecting neurones. A similar pattern of distribution is exhibited in TSEs (97).

In 1927 Ashizawa found histopathological changes in the pons, the internal capsule, and cerebral peduncle (100). In 1936 Trendtel reported degeneration and gliosis in the corpus striatum, in addition to neuronal loss in the putamen and globus pallidus. He likewise found similar changes in other areas of the brain (101).

In 1934 Canavan et al., amongst others, reported atrophy/neuronal loss in the cerebral cortex – especially in the frontal lobes and in the cerebellum – and major changes in the basal ganglia which were shrunken and distorted as normally observed in Mn intoxications (102). Histopathological studies revealed gliosis and degeneration of nerve cells, particularly in the optic thalamus, globus pallidus, lentiform nucleus, caudate nucleus, subthalamic nucleus and putamen.

After improvements in health and safety in the various occupations involving exposure to Mn in the later half of this century, studies into the pathology of Mn intoxication were largely phased out. Unfortunately, microscopic surveillance technology and knowledge of membrane proteins was not sufficiently sophisticated at that time to identify the presence of prion and amyloid plaque tombstone structures characteristic of prion disease.

Banta et al. (6) carried out a more recent pathological study in 1977 involving a case of Mn intoxication associated with dementia and extrapyramidal signs in a patient from rural Kentucky. Micrography of a biopsy of cerebral cortex revealed amyloid plaques composed of bundles of fibrils, lipofuscin granules and argyrophilic neurofibrillary tangles – tombstone features that are characteristic of both TSEs and Alzheimers (129).

Apart from Banta's pathological study (6) making reference to .34 ppm levels of Mn (0.17–0.29 normal) in a frontal lobe biopsy of a single CJD sufferer as part of their control, no work has intentionally set out to investigate for any association between Mn or other cations and TSE aetiology.

Furthermore, transmission studies utilising CNS homogenate from fatalities of chronic Mn poisoning have apparently never been attempted. Such a trial would represent an ideal first step towards eliminating or demonstrating the possible role of Mn or other cations as the transmissible entity in prion diseases.

As this theory suggests that trivalent Mn serves as the transmissible agent in sporadic TSEs, then any Mn 3+ induced neurodegenerative disorder should be transmissible via inoculation of diseased CNS homogenate whether PrP is involved as an Mn3+ conjugate or not.

In contexts of Mn overloading, Mn3+ may be capable of competing and conjugating onto a range of other compatible metallo / cuproprotein ligands, particularly when that protein's normal metal co-partner is in short supply.

Interestingly, possible evidence for the lack of necessity of a 'prion' based infectivity in TSEs was demonstrated in TSE transmission studies where brain homogenate sourced from TSE diseased mice successfully transmitted TSE into recipients. However, various CNS sections taken from these recipients failed to exhibit the presence of 'prions' (95).

**The clinical perspective of Mn intoxication**

Chronic Mn toxicity first reared its insidious, ugly head in the Byzantine era, when Black Magic was referred to as ‘Mangania’ – the term for Mn ore (3). Mn psycho-neurotoxicity was subsequently recognised within the more mainstream world of medicine when those occupationally exposed to Mn ores in the mines of Chile, India, N Africa, N America, the Isle of Guam and Japan developed a bizarre delayed neuropsychiatric condition known as ‘locura manganica’ or ‘manganese madness’ between 1–24 years after starting work in the mines (3). The initial psychiatric phase of the disease was invariably followed by an irreversible progressive neurodegenerative phase; the two stage syndrome becoming widely accepted as the characteristic clinical scenario encountered in chronic manganese intoxications of miners exposed to Mn dioxide dust (97, 104, 105, 11).

Interestingly, the sequence and types of symptom exhibited in the class of Mn intoxication encountered in miners bear close resemblance to the new strain CJD/BSE/Kuru types of TSE (41, 106, 107), whilst the other less common class of Mn intoxication exhibited by workers in the ferro-manganese plants of North America (105, 108, 11, p. 20) does not present with such a clearly defined, broad-ranging psychiatric phase at the onset of symptoms – a clinical profile more akin to the symptomology of sporadic TSEs (41).

Symptoms such as cortical blindness and convulsions which characterise the later stages of sporadic CJD (although absent in the Kuru TSE) (41) do not appear to have been reported in any publication covering Mn neurotoxicity despite reports of upper cortical involvement in the pathology and symptomology of several case studies.

However, epileptic convulsions have been associated with the clinical manifestations of CNS Mn deficiency (4), suggesting a pivotal role for Mn in maintaining neurochemical homeostasis. It is possible that an abnormal CNS accumulation of an Mn 3+/Mn 4+, or simply an Mn2+ species, within a Mg2+ deficient environment (4) (as recorded in TSE regions (Table 1)) could lead to the inactivation of the Mg/Mn dependent enzyme glutamine synthetase and/or a break down in the stabilisation of its molecular conformation (109,4) (glutamine synthetase is essential for converting neurotoxic glutamate into glutamine inside the astrocytes (7)), thus leading to disruption of the neurochemical equilibrium in the frontal zones, which, in turn, increases the risk of convulsions.

Common symptoms of chronic Mn intoxications (3,11,97,104,107) are duplicated in TSEs (41,106); e.g. asthenia, loss of balance, cough, hypsosomnia, insomnia, anorexia, olfactory crises, loss of libido, forgetfulness, lack of concentration, depression, irritability, confusion, headache, unexplained generalised pain, slurred speech, mutism, emotional lability, weeping, excessive laughter/childish smiling, euphoria, salivation, facial seborrhea, excessive sweating, delusions, withdrawal, hyperactivity, paranoia, hallucinations, psychoses/insanity, immaturity, compulsive actions, clumsiness, self neglect, aggressiveness, hypoknesia, rigidity, tremors, upper and lower motor complaints such as aphasia, dystonia, choreathetosis, muscle cramps, speech disturbance, abnormal swallowing or cock-walk gait and postural reflexes, myoclonic jerks, rigidity, cogwheel on the wrists, ataxia, and, neurological disturbances involving a combination of pyramidal, extra pyramidal and cerebellar symptoms.

The common occurrence of somewhat unique symptoms such as ‘unmotivated spasms of laughter/childlike grinning’ in both Mn intoxications (3,97,104,105) and nv CJD/Kuru (41,107) is interesting. Indeed, kuru has been described as the ‘laughing death’ (107), whilst the entire workforce of some Mn mines have been reported to burst into prolonged bouts of unmotivated laughter (105).

There is also variation in the period between initial occupational involvement with Mn and the emergence of the clinical phase of the disease. Miners may develop the initial symptoms of Mn intoxication between one and
twenty four years after first becoming occupationally involved with Mn (97,104,105), whilst others do not develop the neurodegenerative phase of the disease until several years after ceasing employment in the mines – at a time when there is considerably less measurable Mn in their systems than the levels found in healthy active miners (105).

It would appear that the Mn factor does not perform any pathogenic role in ‘propelling’ the secondary neurodegenerative stages of the disease. This is supported by the fact that therapy with an Mn chelator during this secondary stage fails to arrest the disease (112). However, Mn chelators can successfully arrest the syndrome during its initial psychiatric stages (112), often leading to permanent remission.

As case histories of Mn intoxication demonstrate that the neurodegenerative phase of the disease progressively worsens over many months without any requirement for further exposure to Mn itself, then this places the Mn type of ‘hit and run’ delayed neurotoxic syndrome as unique amongst all other recognised types of metal poisoning.

Whilst such a scenario of self perpetuation indicates the presence of free radical chain reactions as the driving force behind the later stages of Mn encephalopathy, it also elucidates possible common pathogenic mechanisms underlying the delayed, so called ‘incubation’ period that are shared by Mn intoxications and prion diseases.

The putative pathogenic mechanisms of Mn intoxication underlying TSEs

The CNS pathogenic mechanisms of Mn intoxication are consequently little understood, although researchers consider that they hinge upon a complex multifaceted series of auto oxidative chain reactions – probably initiated via Mn’s highly reactive trivalent species, Mn 3+, which has been shown to readily oxidize catecholamines ‘in vitro’ (7,29,11).

Such a putative pathogenic mechanism encompassing Mn initiated radical reactions explains the widespread disruption of several classes of CNS receptor, Ca channels, signal transduction cycles and second messenger synthesis that has been noted in chronic Mn intoxications (7). Mn also accumulates in the mitochondria during intoxication where it disrupts the homeostasis of Ca channels raising intracellular levels of free Ca invoking further oxidative stress (7).

Interestingly, Mn2+ also competes with Mg 2+ for the site on the ATP complex. The Mn-ATP bond is strong, thus explaining how Mn displaces catecholamines from their bonding to the ATP complex in the storage vesicles of chromaffin granules in the adrenal medulla (110,3).

80% of the total Mn found in the brain is tied up in activating the manganoenzyme glutamine synthetase which is found exclusively in the astrocytes (7); inferring that abnormal radical reactions derived from Mn 3+ overloading in the CNS would largely remain confined to the astrocytes.

Interestingly, Brown et al. have demonstrated that type 1 astrocytes express PrP relatively intensively (111). Astrocytes are invariably activated in response to proliferating microglial cells during the early stages of TSE (111). The failure of astroglial cells to respond correctly in TSE suggests that the conformational development of PrP within PrP expressing type 1 astrocytes has been corrupted (eg; due to a putative Mn3+ substitution at PrP’s Cu domain), leading to a break down in the regulation of oxidative stress by SOD1 in astroglial cells. Brown et al (111), has demonstrated that the activation of astrocytes and microglial is an essential prerequisite of TSE pathogenesis (NB; astrogliosis is a fundamental pathological feature of TSEs (41)).

Glutamine synthetase performs a vital role in catalysing the conversion of the excitatory amino acid glutamate into glutamine after it has been carried from the synaptic cleft into astrocytes by a high affinity uptake system (7). Interestingly, the conformational stability of glutamine synthetase is regulated by a complex equilibrium involving Mn 2+ and Mg 2+ (109). This elucidates a further putative neurotoxic mechanism stemming from Mn overload, where an excess of trivalent Mn in the astrocytes (coupled to the additional complication of Mg deficiency recorded in TSE ecosystems) (Tables 1 & 2) disrupts the biochemical pathway that mediates the structural stabilisation and activation of glutamine synthetase, leading to an abnormal accumulation of the highly neurotoxic glutamate instead of its normal metabolic conversion product glutamine. Glutamate is excitotoxic to neuronal membranes and various ionic channels which increases intracellular levels of free Ca leading to a variety of radical cascades.

Interestingly, glutamate overloading plays a significant role in the pathogenesis of membrane disruption/degeneration manifested in TSEs (41,89,96) and other neurodegenerative diseases, such as ALS, where glutamate metabolism (113) and its uptake at the glutamate AMPA/Kainate receptors are both impaired (114). Mn 2+ substitutes for Ca at the Ca channels and is recognized as the most potent inhibitor of kainic acid binding to forebrain membranes (115).

Other biochemical facets of TSE pathogenesis such as the increased capping of cells with the lectin, concanavalin A (19), and the 3 1/2 fold increase in the surface expression of PrP invoked by the lectins, concanavalin A and phytohaemaglutinin (18), could be explained by the key role that Mn performs in activating...
these lectins (116,117). Mn activation of lectins like concanavalin A acts as a critical prelude to the subsequent binding of calcium to this lectin, as well as to the subsequent interaction of the lectin with its specific cell surface glycoprotein target (117). Removal of Mn abolishes the glycoprotein binding properties of most lectins.

Once levels of available Mn exceed its normal threshold in the CNS, Mn could overactivate increased amounts of lectins like concanavalin A; a lectin which is known to interact with the membrane glycoprotein PrP (18). Con A is also known to bind to glycosaminoglycans (118); unbranched polysaccharides that are well recognized to bind to PrPc, protecting the protein against bonding to the abnormal PrPsc, which, in turn, appears to protect the PrPsc ‘infected’ mammal against the development of clinical TSE (5). An overloading of Mn could therefore account for yet another crucial primary role in TSE pathogenesis by overactivating lectins that bind to glycosaminoglycans, which, in turn, inactivates the ability of these glycosaminoglycans to bond to PrPc, thus breaking down the protective mechanism designed to safeguard PrPc against its putative lethal conjugation onto PrPsc.

The quantity of lectins in the animal diet could also present a TSE risk factor. Lectins are relatively stable, heat resistant, naturally toxic proteins found in peanuts, beans (locust, kidney and haricot), soya, alfalfa, rice bran, peas, lentils, wheat, bulbs, snails, etc. Interestingly, the majority of these foodstuffs were incorporated into the concentrated rations of UK dairy cattle during the BSE era (119). In particular, the temporal dynamics of annual UK usage of field beans in animal feed rations (119) correlates with the temporal dynamics of annual incidence rates of BSE in the UK (see Fig. 8). A total of 37 800 tons of field beans were used in animal feed rations in 1984, rising to a peak of 247 400 tons in 1989, then dropping to 85 600 tons by 1995.

Once TSE-susceptible animals are dependent upon food supplies that are simultaneously high in both manganese and lectin content, the risk of developing TSE could be significantly increased.

The aetiological association of Mn overloading with other neurodegenerative diseases

Some researchers consider that the secondary irreversible neurodegenerative stages of chronic Mn intoxication is a form of Parkinson’s disease (PD) (11). Despite the similarities, the pattern of pathological damage in most cases of Mn intoxication deviates from the exclusive extrapyramidal pathology of PD. Furthermore, Mn pathology rarely encompasses the substantia nigra (11,97) – the ‘nidus’ of neurodegeneration in PD. However high incidence rates of amyotrophic lateral sclerosis (ALS) and PD have both been recorded amongst Mn miners on Guam and in Japan (115,120,121). Serum Mn levels are frequently elevated in ALS/PD victims (14,122,123), and have been recorded more recently in the serum of those suffering from psychoses, rheumatoid arthritis and Alzheimer’s dementia (4, p. 189) (6) – conditions which have been theoretically associated with a prion induced pathogenesis.

Yase had analysed the soils/plants in the renowned South Pacific cluster of ALS/PD/MS/Alzheimer’s type dementia on Guam, the Kii Peninsula (Japan) (115, 120) and West New Guinea and found that high levels of the divalent cations, manganese/aluminium were recorded in all regions.

Spencer et al. (124) hypothesized on the presence of a key environmental trigger factor underlying the pathogenesis all of these neurodegenerative diseases in the South Pacific cluster, but they plumped for a neurotoxic excitatory amino acid in the natives’ diet of cycad fruit as the putative causal agent operating within a multifactorial
aetiological template, having rejected the possibility that the high levels of Mn/Al cations found in the indigenous terrain could accumulate in cycad fruit and chronically intoxicate the natives causing neurodegenerative disease to surface in later life.

Interestingly, there is a high incidence clustering of MS and ALS amongst some of the subsistent farming communities who used to live directly ‘off the land’ within the Mn-rich scrapie endemic regions of Iceland (125).

Spencer et al. (124) and Gajdusek (126) have elucidated common pathogenic denominators between PD, ALS, AD, etc., suggesting a common environmental trigger factor shared by all of these diseases.

Some of the pathological similarities noted in ALS, AD, and PD are also observed in TSE pathology (129,41). Furthermore the pathogenesis of ALS demonstrates some specific biochemical facets that are shared by TSEs; such as a high turnover of the Mn-containing metalloenzyme (3,4) arginase (127) in the primary stages of pathogenesis of both diseases. High levels of available Mn may ‘switch on’ the increased expression of arginase in the early stages of these diseases. Interestingly, a strain of ALS was induced in young calves following intracerebral inoculation with PrPsc CNS homogenate (128), whilst a specific class of CJD known as atypical CJD exhibits a pathology which combines idiosyncratic features from both ALS and CJD (120, p. 318). Other CJD cases have combined Alzheimer’s neurofibrillary tangles or Parkinson’s Lewy bodies with the usual TSE features (130).

Whilst some researchers have putatively linked cations such as manganese, aluminium and calcium to the aetiology of these neurodegenerative diseases found on Guam and elsewhere across the world, Mn intoxication has hitherto never been previously associated with TSE pathogenesis.

This theory proposes that cations such as Mn or Nickel, in their trivalent context, may play a primary role as the infectious transmissible agent in the aetiology of TSEs, providing the other essential TSE prerequisites are fulfilled, e.g. TSE susceptible genotypes being chronically exposed to Mn/nickel during Cu/Fe deficiency states. Conventional neurodegenerative diseases, as apart from TSEs, will develop in contexts where the cation-overloaded individual possesses optimum levels of Cu in their CNS; thereby ensuring that an adequate supply of Cu occupies PrP’s copper domain, thus protecting PrP against invasion by foreign cations and the resulting induction of an abnormal conformational change of PrP.

**Sources of Mn in the human food chain**

Chronic cumulative Mn intoxication of the general population may result from habitual ingestion of certain foodstuffs or mineral supplements (6) naturally high in the element: e.g. tea leaves (610 p.p.m.), coffee (20 p.p.m.) (especially beans sourced from the Congo), cloves (263 p.p.m.), thyme (82 p.p.m.), brazil nuts (28 p.p.m.), pecans (36 p.p.m.), soya beans (35 p.p.m.), red wine (14 p.p.m.), etc. (3,4).

Bioavailability of Mn is higher in some foods such as soya (70%) than in others like oilseeds (50%) (4).

Exposures to Mn via its industrial applications poses a much greater toxicologically threat to human health; e.g. intranasal exposure to atmospheric fall out of Mn resulting from its use in steel, explosives, glass and dry battery manufacture (3,4,94,108), as a contaminant of cement kiln fuels, and its use as a tricarbonyl Mn additive in unleaded petrol (3). Mn may also contaminate water supplies as a result of its industrial or natural applications (3,4).

Mn also pollutes the atmospheres and food chain due to its use as a fertilizer spray and as an active ingredient in two widely used dithiocarbamate fungicides called maneb (28) and mancozeb/manzidilan (98). A few countries apply these fungicides onto growing farm and garden crops such as blackcurrants, potatoes, tomatoes, apples/pears, hops, wheat, roses/tulips, whilst a very few countries apply them as post harvest fungicidal dip treatments, for protecting strawberries, bananas, tobacco, etc (17,28,130) after harvest to prolong shelf life.

UK usage of Maneb in tonnes per annum increased from 142 tonnes in the late 1970s, to 233 tonnes in the early 1980s to 679 tonnes in 1984 (132,133), then dropped to 297 tonnes in 1994 (133). The temporal dynamics of maneuse usage in the UK correlates to the temporal dynamics of BSE incidence in the UK (Fig. 8). With Maneb ranking at No 13 and mancozeb at No 4 on the top fifty list of overall tonnage of pesticide used in the UK in 1994 (133), fears have understandably been raised concerning the residues of these compounds arising from their widespread use as post harvest fungicide treatments.

Whilst the tonnage of maneb used in 1994 had dropped by 56% since 1984, the tonnage of mancozeb had increased by 235% in this period (133). Many other countries have banned or restricted use of these compounds on the basis of their ability to invoke teratogenic effects such as hydrocephalus in the fetus (134), and a delayed Parkinsonian-like neuropsychiatric degenerative disease in workers chronically exposed to these chemicals (28).

**BSE/nv CJD: a synthetic, synergistic means of invoking the same CNS mineral disturbance underlying the aetiology of sporadic TSEs?** (Fig. 9)

Mn permeated the UK’s bovine food chain in the 1970s/1980s largely as a result of the widespread incorporation of chicken manure into the concentrated feed...
rations of cattle (119), where it was used to bind as well as increase the protein content of the feed. When MBM was banned in 1988, chicken manure was one of the cheaper sources of protein used to replace it. Its use subsequently increased for a short while until it was banned in 1991 (119) – at the peak of the UK’s BSE incidence rate (Fig. 8). Poultry were fortified with various Mn complexes (Mn sulphate, Mn oxide, etc.) for promoting egg and broiler production as well as rearing. Mn was generally fed at high rates between 100 and 120 mg/kg of dry matter composition of diet (135) because of the inefficient 2–5% rate of dietary absorption of Mn by monogastric poultry (135). Consequently, 95–98% of the Mn content of poultry feed is excreted in the manure.

Cows were also increasingly exposed to foods high in Mn via various cost-cutting byproduct ingredients that were added into cattle concentrate feed during the 1970s/1980s (135); palm kernal meal (164 p.p.m. Mn), wheat bran (122 p.p.m.), rice bran (260 p.p.m.), soya bean meal (35 p.p.m.) tea waste (275 p.p.m) coffee waste (20.6 p.p.m) red clover (158 p.p.m.), dried alfalfa (37 p.p.m.) (3,4,69). Nickel was used to extract certain types of oil (4) that are added to concentrated feeds could have entered the bovine food chain.

Concentrated Dairy Cow feed was also supplemented with high rates of Mn at 120 mg/kg (calves at 80 mg/kg) whilst beef cattle that received considerably smaller quantities of concentrated feeds than dairy cattle were only fed 70 mg/kg (135). (NB. Beef cattle experienced 80% lower rates of BSE relative to dairy cattle) Ruminants absorb Mn more efficiently than monogastrics, with 10–18% Mn traversing the gut wall (135).

Exposure to Mn residues resulting from the use of Mn fertilizers and ‘maneb’/‘mancozeb’ fungicides on fodder and forage crops for cattle also occurred – particularly during the period of peak usage of these compounds in the late 1980s/early 1990s (131–133). Any Mn originating from these various sources would also survive the rendering process of meat and bone meal (MBM) manufacture, and subsequently bioaccumulate its way up the farm animal foodchain as a result of the practice of feeding farm animal back to farm animal via the MBM ingredient.

Alternatively, there are two other candidate cations which were used significantly in UK agriculture and along UK waterways. Diquat and Chloramquat cations (related to the auto oxidising paraquat molecule (17)) were used more intensively in the UK by weight per acre during the 1980s–1990s than in other countries. Diquat is applied as a crop desiccator in the UK (17,130) where it is used on peas, grass crops for seed, laid cereals, oilseeds, hops, lucerne, potatoes, beans, etc, immediately prior to harvest (and as a herbicide along waterways), and chloramquat is applied as a plant regulator on cereals shortly before harvest. Diquat use (Graph 3) increased by 800% from 20 tonnes of a. i. used per annum in the later half of the 1970s (131) to 163 tonnes used per annum in the
Ecosystems supporting TSEs demonstrate excesses of the pro-oxidant manganese and Cu, Se, Fe, and Zn deficiencies

early 1980s (132). Usage subsequently dropped and stabilised thereafter, running at 87 tonnes applied in 1984 and 104 tonnes in 1994 (133). Chloramquat use in the UK has increased more dramatically, starting at 239 tonnes per annum in the late 1970s (131), 231 tonnes per annum in the early 1980s (132), then 1112 tonnes per annum in 1984 rising by 110% to 2335 tonnes in 1994 (133) – the highest ranking pesticide in UK usage terms when related to total area treated.

The ‘paraquat’ class of cations are absorbed into plants (17) after application. They remain persistent in specific environments such as clay soils (134). Paraquat forms stable free radicals (17), crosses the blood/brain/barrier in mammals and has been associated with the aetiology of Parkinsons disease and the induction of auto-oxidation of dopaminergic/serotonergic neurones (10,13,14). The possible binding of diquat to the copper domain of PrP during CNS Cu deficiency should also be considered alongside the possible involvement of other candidate cations, such as Mn or nickel (as a more reactive oxidative species; eg Mn 4+ or radioactive Mn) in the aetiology of the ‘modern’ strains of TSE.

**Exposures to synthetic estrogen/steroid compounds accelerate the absorption and accumulation of Mn in the CNS: a putative prerequisite in the aetiology of BSE/nv CJD**

Individuals who are low in copper or iron demonstrate a markedly increased absorption of Mn (3,4). Any increases in exogenous/endogenous sources of estrogen (54) or glucocorticoid (137,138,139) also mediates a dramatic increase in absorption, concentration and distribution of Mn within the organism. When Panic et al (54) administered estrogen to laying hens, Mn levels were elevated 15–70 times higher than those found in the untreated controls.

Elevated levels of steroids/estrogens are known to increase the permeability of the blood/brain barrier's microvasculature to macromolecules (141,142), and this may partly explain the increased uptake of Mn into the CNS following increased corticosteroid turnover.

The following groups are therefore placed at a higher risk of increased Mn uptake as a result of their exposures to above average levels of estrogens/steroids; Adolescent (143) and pregnant (144) females, those prescribed the contraceptive pill, hormone replacement therapy, body building steroids/growth hormone, etc, or those directly exposed to high levels of synthetic estrogenic pollutants derived from detergents, pesticides, cosmetics, phallates, etc, or indirectly exposed via residues in recycled water supplies/foodstuffs, or via habitual ingestion of estrogenic foods such as soya which contains the naturally occurring estrogen, genistein (69). Naturally occurring estrogens are also at high concentrations in some leguminous crops such as lucerne and clovers (69), and it is interesting that lucerne comprised the staple diet of a greater majority of the captive and wild deer and elk in Colorado (57) and the ostrichs and other zoo animals that have succumbed to TSEs.

The practise of using oestrogenic/progesterone hormones for synchronising the heat period and conception in UK dairy herds during the 1980s/1990s could have accelerated the absorption of Mn in the herds which adopted this therapy.

Whilst estrogenic growth promoters have been used in many countries worldwide to boost the final fattening stages of beef cattle (banned since the mid 1980s in the UK), all of those animals treated are invariably slaughtered as three year olds before they have had sufficient time to incubate and manifest the clinical stages of TSE.

However, countries that administered these lipophilic hormones intensively to several species of farm livestock (as well as recycling these hormones back into cattle via feeding of the tallow fraction of MBM feed derived from the slaughtered carcases of hormone treated animals) could have contributed to the potential risk of chronic Mn overloading in their herds.

The tallow fraction of MBM carries the lipophilic contaminants present in the feed. Tallow remained in UK MBM feed once the practise of solvent extraction was stopped by the rendering industry in the early 1980s (145). Further estrogenic substances – albeit naturally occurring (69) – permeated the bovine food chain due to the increased feeding of cheaper protein sources such as soya and alfalfa during the 1980s (119).

Cattle and humans drinking in areas where water is drawn from sources polluted by synthetic estrogenic pollutants could potentially absorb Mn at an increased rate.

Overloading with estrogens/steroids can also depress Cu absorption (36) which, in turn, activates the mobilisation of any remaining Cu stores in the liver; thus increasing the turnover of ceruloplasmin (84) in the hepatocytes which could favour an increased oxidation of Mn2+ into its lethal Mn3+ species (7) – particularly at a time when supplies of ceruloplasmin's normal oxidative target, Fe2+, are depleted.

The common thread centres on the role of these hormones in activating the pituitary-adrenal axis, whereby mediating an increased turnover of the adenocorticotrophic hormone (ACTH) stress response, which increases absorption and hepatic release of Mn as well as increasing permeability of the blood brain barrier (137–139). This permits an increased entry of Mn into the CNS, explaining why higher levels of Mn are found in the serum/whole blood and CNS during times of ‘stress’; eg, during infections, myocardial infarction, rheumatoid arthritis, psychosis, etc. (3) (4), and following surgery.
Other cations, such as nickel, are also found at high serum concentrations in these contexts (140).

Interestingly, a number of TSE epidemiological studies have described these same conditions as common predisposing factors of CJD (41, 146–147, 175) whilst stress events are well recognised to predispose the onset of the clinical phase of BSE. However, authors have invariably attributed any aetiologic association between surgery and CJD to horizontal transmission of an ‘infectious agent’ (41).

The extensive literature citing nickel mediated allergic reactions invoked as a direct result of using orthopedic prostheses, depth electrodes, cardiac valves, surgical instruments, steel sutures or intravenous cannulae composed of nickel alloys (3, p. 220–221) may offer an alternative explanation for the wide variety of surgical procedures which predispose to CJD (175, 41). Following exposure of any PrP expressing tissues to these nickel based devices, nickel could theoretically conjugate onto PrP in susceptible genotypes and initiate TSE.

The association between growth hormone therapy with extracts of pituitary and ‘iatrogenic’ CJD has also been ascribed to horizontal transmission of the CJD agent (41). Interestingly, Mn is well recognized to concentrate in the mitochondria-rich pituitary gland (149), and it should also be noted that long-term therapy with extracts of pituitary growth hormone is invariably administered in conjunction with steroids, where both treatments would simultaneously activate the pituitary–adrenal axis and upregulate ACTH mediated Mn absorption/release (137–139).

Interestingly, anecdotal indications of associations between CJD and steroid/hormone replacement therapy (HRT) medication have been demonstrated in a few contexts, although these incidences would be expected; given the significant proportion of middle-aged/elderly people who are prescribed steroids and HRT, etc. Long term therapy with steroids was reported in two cases of Nv CJD. A proportionately large number of ‘body builders’/gymnasts have contracted Nv CJD.

**Chelation of copper in the CNS by organo dithiophosphate insecticides as a prerequisite of BSE?**

It is proposed that the UK’s unique mandatory high dose usage of a systematically formulated 20% concentrated organo phthalimido-phosphorus insecticide (phosmet) for the control of warble fly on cattle during the 1980s (2, 150) depleted the supply of available copper within the bovine’s CNS; thus depriving the Cu domain on PrP (157, 41) and other cuproproteins (such as the amyloid precursor protein) of a supply of available Cu, causing PrP to loose its correct conformation. The vacated Cu domain becomes vulnerable to invasion by other foreign cations present in the farm animal food chain (e.g. Mn3+, Ni3+ or diquat) that can compete for binding to histidine imidazoles, and theoretically transform PrP into its ‘infectious’ TSE-isoform; the misfolded PrP-cation complex.

BSE runs at its highest incidence rate per total head of cattle in counties (151) such as Hampshire, West Sussex, Norfolk and Wiltshire where the Cretaceous terrains and sandy soils are renowned for their low copper status (31); suggesting that an underlying environmental deficiency of Cu may exacerbate this putative prerequisite of susceptibility to TSE, as recorded in the environments supporting sporadic TSE clusters.

Cu deficiency is also prevalent in the soils of Guernsey Island (31); an area which hosts the highest incidence rate of BSE in the world (151). Brittany is one of the most noted ‘hotspots’ of Cu deficiency in France, and has suffered 20 of the total of 28 French BSE casualties recorded to date.

The underlying problem of Cu deficiencies indigenous to certain UK regions was exacerbated post 1982 when it first became mandatory to treat cattle with the Cu-chelating phosmet based insecticides during the late March period (152) – at a time when copper levels are already at their lowest level in the seasonal cycle (8).

Interestingly the few other countries outside of the UK (namely France and Ireland) who have employed the high dose ‘systemic’ warblecide brands containing 20% phosmet – albeit voluntarily and less intensively – suffer from a relatively less intense incidence of BSE (150).

A phthalimido-N-methylmercaptan (PNMM) group is yielded during phosmet metabolism following hydrolytic loss of the alkyl phosphate moiety (153). It is proposed that PNMM chelates available copper ions in the CNS – much like mercaptotoethanol/dimercaprol (154) – employing its two sulfur or nitrogen to form a tight stable complex with copper which participates in the formation of a mercaptide ring. PNMM subsequently protects itself against further enzymic mediated degradation due to the occupation of its Sulphur catalytic centre with Cu ions. Some of the ‘stable’ lipophilic Copper-PNMM complex is trafficked like a ‘trojan horse’ into CNS lipid membranes (perhaps initiating lipid peroxidation and radical reactions), whilst the remainder is excreted.

A tenfold upregulation of the surface expression of PrP was invoked after Whately et al introduced 12 ppm doses of phosmet into neuroblastaoma cell cultures expressing PrP (155). Such a tenfold upregulation of the cuproprotein PrP ‘in vivo’ would place further demands upon already depleted supplies of free copper in the neurone, leading to the eventual failure of PrP to maintain its tertiary conformation, plus a failure of PrP to perform its putative role in delivering Cu to the superoxide scavenger, SOD 1 (40).

Incidents of poisoning with some types of OP compound have produced the occasional case of spongiform encephalopathy with gliosis in exposed individuals (156,
suffering from the same ‘strain’ of TSE. Nv CJD and BSE are either indirectly related; in that they have originated from an independent exposure to the same environmental agent, or they are directly related; in that humans contracted nvCJD from horizontal exposure to ‘prions’ via a route of ingestion or vaccination with BSE affected bovine CNS tissues.

With only 36 total cases of nvCJD reported to date, plus very limited collated data of the medical histories, occupations or spatio-temporal epidemiology of these cases in the public domain, it is virtually impossible to draw any scientifically based conclusions.

However, many of these cases have emerged as mini focal clusters in rural pockets with cases occurring in adjoining villages – similar to the rural foci characteristic of sporadic CJD (41, 173, 61, 147, 148, 62, 174, 175, 176). The most renowned cluster involves seven cases to date that are centred in the Weald district of mid Kent, SE England. Interestingly, the Weald is a unique agricultural district in that hops and top fruit are intensively grown there. According to a survey carried out in 1980 (177) by the Hop Farmers Association, hop farms and orchards adjoin all of those villages which have been associated with cases of nv CJD – High Haldon, Mersham, Bethersden, Sissinghurst, Tonbridge.

Mn fertilizer sprays and cation-based pesticides such as maneb/mancozeb/diquat and systemic Cu chelating organo dithiophosphates are used more intensively on hops and fruit than on other types of crop in the UK (131, 132, 178, 179). According to MAFF pesticide usage surveys (131, 132), systemic OPs were used 100 times more intensively on hops during 1983, in kg per acre per year, than on the common arable crops grown all over the UK. This potentially places the levels of pesticide particulates in the atmospheres of the Kentish Weald valleys during the spray season at the highest ranking in the UK.

An interesting paper demonstrated a high incidence rate of sporadic CJD amongst market gardeners and farmers in Australia, where 83 out of a total of 241 confirmed CJD cases belonged to this occupational category. (174)

All surveys to date have only investigated the unproven, unilateral assumption that new variant CJD is caused by ingestion of bovine tissues infected with BSE. Whilst this represents one important direction that has to be addressed, surveys should also investigate the wider perspectives of the environments surrounding these focal clusters, so that equally important questions raised by the various alternative theories are properly investigated.

ACKNOWLEDGEMENTS
My gratitude is extended to the following for their generous assistance in providing vital contacts / answering queries, etc. regarding the epidemiology of the respective TSE clusters in their country. Dr Eva Mitrova, Institute of Preventive and
Clinical Medicine, Bratislava, Slovakia. Dr Sigurdur Sigurdarson, Central Vet Laboratory, Keldur, Iceland. Professor Beth Williams, Department of Veterinary Science, University of Wyoming, Laramie, WY, USA. Dr Mike Miller, Colorado Division of Wildlife, Fort Collins, Co, USA. Also to Dr David Brown, Department of Biochemistry, Cambridge University, UK, for encouragement and enlightening discussion.

REFERENCES


44. Kristensson K., Feuerstein B., Taraboulos A., Hyun M., Prusiner S., DeArmond S. Scrapie prions alter receptor-mediated...
Ecosystems supporting TSEs demonstrate excesses of the pro-oxidant manganese and Cu, Se, Fe, and Zn deficiencies.


95. 'Usage of raw materials in Animal feeds in Great Britain 1984–1995'; or personal letters – Alex Clothier, MAFF Statistics, Foss House, Kings Pool, 1–2 Peasholme Green, York, Y01 2PX, UK.


