Medical Hypotheses (2005) x, xxx–xxx

ELSEVIER

medical hypotheses

http://intl.elsevierhealth.com/journals/mehy

Metal microcrystal pollutants; the heat resistant, transmissible nucleating agents that initiate the pathogenesis of TSEs?

Mark Purdey *

High Barn Farm, Elworthy, Taunton, Somerset TA4 3PX, UK

Received 8 March 2005; accepted 9 March 2005

Summary This paper exposes the flaws in the conventional consensus on the origins of transmissible spongiform encephalopathies (TSEs) which decrees that the protein-only misfolded 'prion' represents the primary aetiological transmissible agent, and then reviews/presents the emerging data which indicates that environmental exposure to metal microcrystal pollutants (sourced from munitions, etc.) represents the heat resistant, transmissible nucleating agents which seed the metal-prion protein (PrP)-ferritin fibril crystals that cause TSE. Fresh analytical data is presented on the levels of metals in ecosystems which support populations affected by clusters of variant Creutzfeldt–Jacob disease (vCJD), sporadic/familial CJD, and the scrapie types of TSE that have emerged in the UK, Sicily, Sardinia, Calabria and Japan. This data further substantiates the abnormal geochemical template (e.g., elevated strontium (Sr), barium (Ba) and silver (Ag)) which was observed as a common hallmark of the TSE cluster ecosystems across North America, thereby supporting the hypothesis that these microcrystals serve as the piezoelectrion nucleators which seed the growth/multireplication of the aberrant metal-PrP-ferritin fibril features which characterise the neuropathology of the TSE diseased brain. A secondary pathogenic mechanism entails the inactivation of the sulphated proteoglycans which normally regulate the mineralisation process. This can be induced by a rogue metal mediated chelation of free sulphur, or by contamination with organo-sulphur pollutants that substitute at natural sulphur bonds, or via a mutation to the S-proteoglycan cell line; thereby enabling the aberrant overgrowth of rogue fibril crystal formations that possess a piezoelectric capacity which compromises the ability of the contaminated individual to process incoming acoustic/tactile pressure waves in the normal way. The crystals transduce incoming sonic energy into electrical energy, which, in turn, generates magnetic fields on the crystal surfaces that initiate chain reactions of free radical mediated spongiform neurodegeneration. Metal microcrystal nucleating agents provide a group of plausible aetiological candidates that explain the unique properties of the TSE causal agent — such as heat resistance, transmissibility, etc. — which the protein-only prion model fails to fulfill. This paper also discusses the possible nutritional measures that could best be adopted by populations living in high risk TSE ecosystems; as a means of preventing the successful implantation of these rogue microcrystals and their consequent hypermineralisation of the soft tissues within the CNS.

* Tel.: +44 1984 656832.
E-mail address: tsepurdey@aol.com.

0306-9877/$ - see front matter
doi:10.1016/j.mehy.2005.03.018

Introduction

The conventional hypothesis on the origins of TSEs

The conventional hypothesis on the origins of transmissible spongiform encephalopathies (TSEs) decrees that these diseases are caused by various modes of exposure to brain tissue that has been sourced from mammals who are infected with a protein-only agent known as the *prion* [1]; a misfolded, protease resistant version of the native prion protein (PrPc), which is a membrane cuproglycoprotein that is expressed in both neural and extra-neural tissues of healthy mammals [1]. Once the abnormal 'prion' isoforms have formed, they tend to aggregate into the metallo-proteinaceous fibril 'tombstone' structures which hallmark the TSE diseased brain [1].

The conventional consensus [1] decrees that an '*infection*' with prions can be contracted via several modes of exposure:

1. Ingestion of prion contaminated CNS derived feedingstuffs.
2. Injections of prion contaminated tissues (e.g., involving blood/growth hormone pituitary tissues).
3. Cranial implantation with prion contaminated depth electrodes, etc.
4. Or as a result of body to body contact (via saliva, etc.).

However, whilst some hard evidence exists to substantiate the fact that TSEs can be transmitted via route 2, and to a much lesser extent via route 1 and 3, there is no evidence in support of the fact that TSEs can be successfully transmitted as a result of body to body contact (e.g., route 4). Furthermore, there is no actual definitive proof which substantiates the idea that the protein-only component of the prion represents the actual transmissible agent.

In fact, a study of the epidemiological history of BSE, scrapie, Creutzfeldt–Jacob disease (CJD), etc., indicates that horizontal transmission of the TSE causal agent is a most unlikely possibility [2,3]. But TSE researchers have jumped to the conclusion that body to body transmission represents the mode of 'infection' whenever clinical disease has simultaneously emerged amongst a localised population group, yet it is just as plausible to assume that the cause of the outbreak may have stemmed from the co-exposure of susceptible individuals to a common toxic denominator that has been introduced into the local ecosystem.

Despite the fact that the conventional consensus on the origins of TSE is founded upon a totally unproven hypothetical assumption, the global community has become virtually indoctrinated that TSEs stem from hyperinfectious origins. The evolution of such a dogma within public consciousness is not surprising, since the population has been subjected to a sustained 'in vogue' campaign of 'scaremongering' over the risks of prion infection resulting from meat consumption in its various guises. Furthermore, the major political pressure groups, the popular press, government/corporate beef trade warmongers, vegetarian campaign groups, etc., have conveniently marshalled the infectious TSE concept into the foundations of their political/commercial agendas. These non-scientific influences have enabled the theory to leap into a position of 'gospel status' without ever being subjected to the rigorous degree of scientific challenge that is usually required prior to the acceptance of any new theory.

But despite the unquestionable endorsement of the prevailing prion theory, there are many blatant flaws [2,3] which blight the viability of the hypothesis and have never been adequately addressed by the proponents of this theory to date. In this respect, the primary causal agent that initiates TSEs remains an enigma. This paper presents the continuation of an investigation into the possible environmental origins of this mysterious group of conditions.

The flaws in the conventional consensus on the origins of TSE

The infectious facet of the prion theory is solely based upon the evidence that TSEs can be transmitted — albeit in the artificial laboratory context — via inoculations of substantial doses of TSE diseased brain homogenate into laboratory animals [1]. Yet, various other diseases, such as familial Alzheimer's disease [4], thyroiditis [5], malignant cancers and toxic metal encephalopathies have also been transmitted in this way.

Since the transmissible capacity of TSEs is shared by these other 'non-infectious' diseases, we need to question why such a high level of health risk has been attributed to TSEs in relation to these various other transmissible diseases? Furthermore, we need to question why it has been assumed that a *transmissible* capacity implies an *infectious* capacity?

In respect of the theory relating to the origins of the modern strains of TSE — BSE and vCJD — there are many radical flaws which discredit the idea that

bovine ingestion of micro doses of scrapie contaminated MBM lead to the cause of BSE [1]. Equally flawed is the follow up theory that human ingestion of BSE contaminated beef caused vCJD [6].

The key Flaws are as follows:

1. Thousands of tons of the BSE incriminated meat and bone meal (MBM) cattle feed were exported out of the UK during the 1960s/1970s/1980s/1990s to countries whose cattle populations have remained BSE-free to date — e.g., South Africa, Sweden, Eastern Europe, Middle East, India, Third World, etc. [7,8].
2. Relaxation in the temperature/solvent extraction of the MBM rendering process in the UK was jointly co-blamed for permitting the survival of the scrapie agent in the sheep brain material [9]; thereby enabling the scrapie agent to 'jump' across into cattle producing BSE. However, none of these alterations were exclusive to the UK rendering plants, since other scrapie endemic countries, such as USA and Scandinavea, had also adopted the same ''continuous flow'' system of rendering; in fact, five years before the UK [10]. Yet these countries have remained BSE-free to date. Furthermore, the pathogenic, 'infectious' capacity of the scrapie agent has been shown to remain active, even after heating up to temperatures in excess of 800 °C [11] — more than five times higher than the 150 °C temperatures that were employed in the supposedly 'safe' rendering processes that operated during pre BSE days up until the late 1970s [9].
3. Several live animal trials conducted in the USA failed to induce BSE in cattle after feeding/injecting them with high doses of brain homogenate sourced from animals suffering from various strains of scrapie [12,13].
4. 43,000 cows that were born *after* the UK's 1988 ban on MBM incorporation into cattle and other ruminant feeds have still developed BSE [14].
5. The cause of these 43,000 *misfit* cases of BSE was partly attributed to the vertical transmission of the infectious prion from mother to calf via the placenta [14]. But *no cases* of BSE could be induced in the 600 calves involved in live animal studies carried out at the UK government's High Mowbray experimental farm; where their mother cows were subjected to high risk BSE conditions (e.g., fed the scrapie contaminated MBM feed) throughout pregnancy [15].
6. Several countries, such as Ireland, Portugal, France and, more recently, Canada, have witnessed a greater number of BSE cases in cows born *after* their respective bans on MBM than in cows born *before* their bans — in accord with the figures published in the UK Department of Agriculture BSE annual reports [14].
7. There have been no reported cases of BSE in other TSE-susceptible ruminants in the UK, such as sheep and goats [14], despite the customary inclusion of the same BSE-incriminated MBM protein source in their feeds [14]. Likewise no cases of BSE have emerged in any cow that was conceived and raised on a fully converted organic farm [16], despite the 'unwitting' exposure of those cows to MBM ingredients as a result of the allowance in the organic standards of 20% of their concentrated feeds stemming from conventional sources.
8. Four of the original five kudu antelope that developed BSE at the London zoo had not had any possible access to feeds that contained MBM [17].
9. The UK government's former experimental farm at Liscombe on Exmoor was designed to raise suckler beef cattle on a pure grass/silage system — without resort to feeding any MBM containing concentrated feeds at all. Yet BSE emerged in four animals on this holding [3].
10. It is customary for Icelandic sheep farmers to slaughter and consume their scrapie affected sheep (including the scrapie affected brains that were consumed in a dish called 'potted head') immediately the first symptoms of this rapid wasting disease are recognised. Yet, no cases of CJD have been recorded amongst Icelandic sheep farmers, and only two cases of CJD in the Icelandic population at large [18,19].
11. The mechanically retrieved meat products/baby foods blamed for causing vCJD in the UK were exported all over the world to countries where vCJD has not emerged to date [3].
12. Many of the aforementioned flaws indicate that BSE fails to fulfill ' Koch's postulates' — the yardstick for gauging whether a given disease (e.g., BSE) stems from a specific infectious agent (e.g., the scrapie agent). One further example of this failure is illustrated by the 10—30% of cattle that were slaughtered each week under the BSE slaughter order, where the presence of the 'infectious' prions could not be identified in the post mortem analyses of their brainstem samples [14]. The identical clinical and spatio-temporal epidemiological profiles of these so called

''BSE negative'' cases [20] in relation to the BSE positive cases, suggests that the ''negative'' cases were suffering from the same disease. The fact that the hypothetical causal agent, e.g., the prion, could not be identified in an average of 18% of the total cattle killed under the BSE order indicates that the official theory fails to fulfill Koch's postulates. Furthermore, research by Lasmezas et al. [21] indicates another failure of the postulate; where laboratory mice developed BSE after being inoculated with BSE affected brain homogenate, yet the presence of abnormal prions could not be detected in the brains of the recipient mice.

These severe flaws in the conventional theory on the origins of BSE suggest that the malformed prion protein is unlikely to represent the primary causal agent. On the other hand, indisputable evidence exists which indicates that the misfolded prion protein performs a critical pathogenic role in the secondary stages of the TSE disease process; where it could be speculated that the loss of function of PrPc (due to its aberrant misfolded conformation), in combination with its mysterious multireplicating property, has been shown to play a crucial role in the progressive neurodegenerative stages of the disease [1].

The alternative theory; the pollutant 'metal nucleator' origins of TSE

In 1998, the author completed a pioneering global expedition which had mapped a geochemical profile of the levels of metals in the ecosystems that support populations affected by high incidence clusters of TSE. The author subsequently published these original observations and his attendant theory that high levels of manganese (Mn) and low levels of copper (Cu) may lead to a Mn substitution at the vacant Cu binding domains on PrPc, and initiate TSE [2]. Work subsequently conducted on PrPc cell cultures at Cambridge University [22] by Dr. David Brown added support to this environmental data and hypothesis; since the results of Brown's experiments demonstrated that Mn bonded to PrPc in place of Cu, and, furthermore, that this rogue metal substitution invoked a transformation of PrPc into its protease resistant, misfolded PrPsc form — the malformed protein that is widely recognised to hallmark the TSE diseased brain [1]. This was the first time that an 'in vitro' transformation of PrPc into its abnormal PrPsc had been achieved as a 'de novo' event.

Various Laboratory and environmental observations [23—27] that have been amassed since the earlier work continue to support the original idea that vCJD and BSE could both result from separate exposure of bovines and humans to a toxic metal environmental factor — e.g., a metal microcrystal nucleating agent [2,3,22,23,25] — and not from the ingestion of the one TSE affected species by the other species. Follow up research by the author suggests that the successful establishment of a rogue metal-protein crystal within the brain renders the individual susceptible to an additional environmental prerequisite involving a secondary exposure to acoustic shockwaves or UV light [3,26,27]. In this respect, the development of misfolded PrP during the TSE disease process could be viewed as a 'de novo' toxicologically induced transformation event, and not as a result of an infection with an exogenous source of PrPsc, which, in turn, induces a *domino-style* conversion of contiguous healthy PrPc to transform into its malformed PrPsc conformation. This de novo transformation is directly related to the knock-on effects of a primary intoxication by these metal nucleating agents — a toxic legacy which pivots upon a foreign metal replacement binding onto vacant copper/zinc metallodomains on prion protein [28] or sulphur domains on proteoglycan heparans. These 'seed' the progressive multireplication of a metal-protein crystal template, and the resulting crystals invoke a diverse array of radical mediated pathogenic complications and TSE ensues.

Does a disruption or displacement of the natural sulphur supply in the CNS represent a critical prerequisite in the development of rogue PrPsc and the subsequent emergence of TSE?

A more recent, broader based environmental analyses of the levels of 46 metals in ecosystems supporting European/Japanese/North American clusters of TSE has established that elevations in certain sulphur-chelating metals, Ba, Sr, Ag and/or Mn [2,27], in combination with deficiencies of natural sulphur (S) and selenium (Se), represents an idiosyncratic mineral imbalance which consistently characterises the TSE cluster ecosystem. Pollutant sources of organo sulphur based pollutants (oil derived, etc.) were also identified in some of the TSE cluster locations, and, if relevant to the aetiology of TSE, these rogue sulphurs may successfully act as substitutes at the vacant sulphur binding domains on PrPc and S-proteoglycan heparans — due to the co-existing deficiencies of natural sulphur recorded in these specific ecosystems.

In this respect, *either* a rogue organo-sulphur replacement binding *or* a rogue metal induced sulphur chelation could represent different means of achieving the same mode of disruption at some stage in the pathway of PrPc/S-Proteoglycan co-metabolism.

Recent studies [29] on the crystal structure of both PrPc and misfolded PrPsc isoforms has identified the di-sulphide bonds as the possible site which hosts the critical modification in the conformation of the prion protein. In this respect, the all important conformational change of PrPc could pivot upon a disruption in the arrangement of the di-sulphide bonds which leads to the development of the dysfunctional PrPsc isoform. These bonds could act as the critical target site where the rogue metals can interact.

Interestingly, the metals Ba, Sr, Mn and Ag that have been observed at elevated levels in TSE cluster ecosystems are sulphur chelating agents [30,31]; thereby indicating that these pollutants could interfer with the development of the di-sulphide bonds on proteins like PrPc, as well as the sulphation of the sulphated proteoglycan heparan groups [32] that are associated with healthy PrPc metabolism; thereby instigating this primary disruptive event in TSE pathogenesis.

This paper presents further analytical data amassed from TSE cluster ecosystems which provides additional evidence that the presence of elevated levels of the metals Ba, Sr, Ag and/or Mn — in combination with other eco cofactors — could predispose the local mammalian inhabitants to an increased risk of developing TSE; thereby playing a primary pathogenic role in the origins of TSEs.

The source of these metal microcrystals has been almost invariably associated with the close proximity of the TSE cluster regions to military controlled sites where munitions have been manufactured, exploded, tested and/or incinerated [3,27], or, alternatively, to various industrial sites (steel, ceramic, glass, welding, rubber, oil and gas drilling, etc.) or volcanoes [2,3]. Interestingly, all of these industrial/natural processes involve the heating of the metallic ingredients to high temperatures, before they are vented out into the open atmosphere as nano particulates. The actual exposure of these metals to the physical effects of the combustion/explosion event has been shown to reduce the overall size of the metal down to the nano particulate.

Individuals who are chronically exposed to atmospheres that are contaminated with these metal microcrystals can absorb them directly into the brain via the nasal-olfactory route of inhalation [33,34], whereas those who are chronically exposed to microcrystal contaminated foodchains could absorb them across the gut/blood and blood/brain barriers [2,35].

Other environmental variables, such as drought conditions, will increase the concentration of the uptake of these microcrystals [27], particularly in respect of 'overpopulated' grazing animals that tend to ingest increased quantities of topsoil particles (and the metals therein [36,37]) during impoverished periods of 'tight' grazing. In this respect, drought periods have been associated with the onset of TSE epidemics in grazing animals, particularly in the regions of North America where Ag and Ba crystals have been utilised as cloud seeding atmospheric sprays for inducing rainfall/suppressing hailstorms [27]. The 'in vogue' overuse of vitamin D3 feed additives in all mammalian species that are affected by TSEs will considerably promote this aberrant hypermineralisation of the soft tissues.

Once these foreign metals have been successfully absorbed and implanted into the brain tissues, they are free to act as pathogenic nucleating agents which initiate an aberrant hypermineralisation of the soft tissues; seeding metal-prion protein-ferritin crystals within the neuronal membranes. These multireplicate themselves over the prolonged 'incubation' period that is archetypal in TSEs, ultimately constructing the abnormal heat resistant crystalline fibril structures [1,38] that characterise the TSE diseased brain.

It has been proposed that these 'fully fledged' crystal formations possess 'piezoelectric' properties [27] which compromise the abilities of the brain to process incoming shock bursts of sound and other pressure energies from the external environment. But this all important pathogenic trigger can only be activated once the crystal contaminated victim is exposed to the specific frequency of sound that matches the specific absorption spectra of the type of crystal [39] contaminant involved. During the so called *incubation period* of the TSE syndrome [1], the crystals grow into full sized fibril structures which lie dormant in the brain until such a time as the contaminated individual is exposed to the specific shockburst of sound which activates the latent pathogenic capacity of these crystals.

Much like the piezoelectric crystals found in the microphone [39], any incoming pressure waves of sound energy find themselves transduced by the crystals into electrical shock bursts, which, in turn, generate magnetic fields on the crystal surface that initiate a progressive free radical mediated chain reaction [40] of spongiform neurodegeneration. TSE ensues (see Fig. 1).

This phenomena is well illustrated by the UK veterinary surgeon's customary 'hand clap' test for 'on farm' diagnosis of clinical BSE. If the BSE suspect

cow responds to the acoustic waves of a gentle hand clap by collapsing to the ground in a state of manic frenzy — as if an electric shockburst had exploded in the poor cow's brain — then a BSE slaughter notice is immediately served on that cow.

Furthermore, 'in vitro' studies have demonstrated that the experimentally induced propagation of abnormal PrPsc in TSE diseased cell cultures will cease when irradiation with ultrasound waves is terminated [41]. This team concluded that the ultrasonic prerequisite performs a crucial role in the breakage of PrP aggregates — one of the key factors which proved to be essential for the propagation of the rogue prion protein [41]. Unfortunately, the full relevance of the sonic effects that have been shown to be critical in these and other 'in vitro' studies have not been cross connected to the *real life* observations of the sonic shock prerequisite that has been shown to characterise the TSE cluster environments [3,27]. In this respect, the possible relevance of a sonic prerequisite in the secondary stages of TSE pathogenesis has hitherto not been realised.

The well known ability of Ba, Sr, Ag or Mn to chelate free sulphur within the biosystem [30,31] — as well as directly binding into the sulphated proteoglycan (S-proteoglycan) centres — could enable this aberrant progressive proliferation of metal-protein crystal growth to proceed in the soft tissues; due to a disruption in the synthesis of viable S-proteoglycans — molecules that would normally act as endogenous inhibitory regulators of mineralisation (e.g., crystal growth) throughout the biosystem [42].

Furthermore, the sulphated heparan types of S-proteoglycan have been shown to play an important associative role in the healthy metabolism of PrPc [43], whilst the loss of activity of these S-proteoglycan molecules has been shown to be associated with the pathogenesis of TSEs [44,45]. In

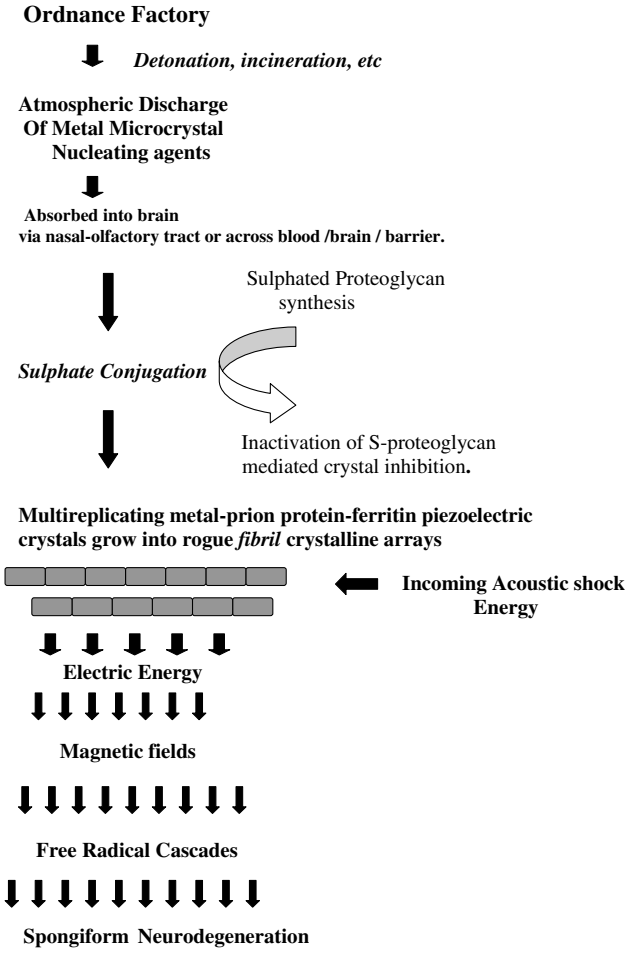


Figure 1 The 'stack up' of piezoelectric pathogenic capacity; rogue metal nucleated protein crystal arrays transduce incoming acoustic energy into electric energy, which, in turn, generates magnetic fields on the crystal surface that initiates a free radical mediated pathogenesis of spongiform neurodegeneration.

this respect, the author has proposed that the copper component of healthy PrPc performs a co-operative role with the S-proteoglycans in a relay-like conduction of the circadian mediated electrical signalling that regulates the turnover of the nerve and fibroblast growth factors, etc. [3].

The elevated levels of Ba, Sr, Ag or Mn free ions entering the biosystem will form rogue sulphate complexes at the sulphur sites on PrPc and S-proteoglycans — for example, by binding into the disulphide bonds and disrupting the critical tertiary folding stages of PrPc and the all important development of PrP's three dimensional conformation [29], thereby seeding the growth of metal-PrP-ferritin protein crystals.

Whilst theories suggesting a role for nucleating agents in the pathogenesis of TSE have already been advanced [46,47], these various hypotheses have always focused on the protein-only 'prion' as the seeding agent. However, this theory is novel in that it marshals original environmental observations that have been recorded within TSE cluster locations and integrates them into a unified proposal which suggests that metal microcrystal pollutants represent the heat resistant, transmissible, nucleating agents that seed the pathogenesis of TSEs.

Methods

Soil sample collection/analysis method

Each soil sample comprised a 300 g sample drawn from a mix of 20 columns of dry soil bored with a stainless steel auger; each column having been bored at equidistant intervals along a W shape spanning an area of ≈5 acres, the area being representative of the region housing/growing crops supporting the TSE affected population under study. Each column was drawn from the top soil to a depth of 6 in. having taken care to avoid inclusion of root material/surface organic matter and collection of samples near to gateways, roadsides, animal dung, disturbed/excavated or polluted terrain. The 20 columns were collected into a plastic bag, then mixed into an even homogenate, from which a further sample of no more than 300 g was drawn and placed into a small polythene bag, then sealed, labelled and transported to the laboratories at the Department of Geology, Royal Holloway, University of London, Egham Hill, Surrey TW20 0EX, where samples were subsequently dried in the laboratory in forced air flow cabinets. The temperature was maintained below 32 °C during the 12 h drying period, whereupon the air was constantly dehumidified. The soil samples were then 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. Each sample was analysed by standard ICP/MS analytical procedure where Al, Fe, Mg, Ca, Na, K, Ti, P, Mn are quoted as weight per cent oxides and the remainder of elements quoted as ppm.

Vegetation/foodstuff sample collection/analysis method

Each plant tissue sample comprised a 200 g sample representing tissue collected from approximately

Table 1 Increases in levels of Sr, Ba, Ag in TSE cluster ecosystems in North America and Europe/Japan in relation to the levels recorded in the ecosystems of adjoining TSE-free control regions

Location	TSE type	Matrix	Sr increase	Ba increase	Ag increase	Mn increase
Sardinia	Scrapie	Pasture	2.4×	3.0×	2.5×	3×
Japan	CJD	Soil	2.8×	1.5×	NR	−1.4×
Calabria	CJD	Soil	2.8×	2.5×	3.8×	1.7×
UK/sicily	vCJD	Soil	3.8×	1.7×	2.0×	7.9×
Sardinia	Scrapie	Soil	2.0×	1.8×	2.1×	Same
Mean increase			**2.7×**	**2.1×**	**2.6×**	**2.2×**
N. America	CWD	Pasture	3.9×	2.0×	2.7×	1.2×
N. America	CWD	Soil	2.0×	1.3×	1.4×	1.2×
N. America	CWD	Antler	2.4×	3.3×	2.6×	−3.0×
Mean increase			**2.8×**	**2.2×**	**2.2×**	**−0.2×**

10 pickings/diggings sourced at equidistant spacings along a W shape (where possible) across an area of ≈5 acres that was representative of the region lived in/harvested by the TSE population under study. Samples were picked dry and away from roadsides, gateways, animal manure, polluted or disturbed terrain, whilst care was taken to avoid inclusion of any root, leaf or soil materials that would not normally get ingested. The tissue was packed directly into plastic bags, lightly sealed, labelled accordingly and transported to the laboratories at the Department of Geology, Royal Holloway, University of London, Egham Hill, Surrey TW20 0EX. Each sample was placed in a plastic sieve and thoroughly washed in deionised water. After removal of any roots or soil, the samples were spread evenly on a drying tray and dried in a 90 °C oven to constant weight, and then ground by Christy Norris mill, a small portion of the next sample being 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). Analyses was by standard ICP scan.

Results

The results are displayed in Tables 2–6. In respect of the 43 metals that were screened in

Table 2 Levels of metals in soils sourced from scrapie cluster and scrapie-free areas across Sardinia [48,53] (Sample collection; October 24th–November 3rd 2002)

Farm location	Mn ppm	Ba ppm	Sr ppm	Y ppm	Ag ppm	U ppm	Th ppm	Cu ppm	Al %	Ca %	Mg %
Sicily											
S Lucia de Mela	859	334	179	22	0.3	2.38	10.9	40	16.8	2.91	2.16
	751	555	285	22	0.3	3.53	12.3	46	15.3	2.26	2.27
	766	645	259	30	0.3	7.95	14.1	84	16.7	1.91	2.98
Sardinia											
Columbu	658	565	105	26	0.1	4.18	20.2	17	14.9	0.91	0.85
Bussu G	356	1369	111	15	0.02	3.08	10.3	4	15.4	0.44	0.81
Bussu C.	557	478	96	12	0.00	2.39	7.90	5	12.9	0.70	0.74
Marchi D.	743	627	169	18	0.01	1.82	8.19	9	15.7	2.12	2.19
Mareo N.	495	606	148	25	0.02	2.11	8.81	8	15.4	1.68	0.85
Musei	689	658	68	19	0.30	2.12	9.76	31	14.9	0.15	1.10
Uta	1037	851	115	23	0.36	2.45	13.4	33	14.5	1.19	1.14
Assimini	673	579	211	24	0.16	1.98	10.3	29	13.3	7.49	1.44
Siamanna	495	557	239	20	0.04	1.84	7.56	11	14.4	5.13	1.04
Silan Birori	937	1027	413	25	0.42	1.13	6.23	37	17.2	2.23	1.34
Silanus	642	669	98	21	0.01	1.73	8.32	19	13.4	1.02	0.68
Ozieri Ochetta	898	559	156	16	0.03	1.48	10.3	14	14.0	1.28	0.55
	744	415	237	30	0.20	2.09	11.4	29	14.6	3.52	2.07
Ozieri W	774	570	121	18	0.19	1.43	9.47	27	15.4	2.25	1.08
Ozieri N.	1091	630	260	16	0.03	4.64	12.8	19	16.0	1.81	0.81
Mean Scrapie	**731**	**650**	**182**	**21**	**0.17**	**2.68**	**10.7**	**26**	**15.0**	**2.17**	**2.41**
TSE-free (Sardinia)											
Fertilia	362	330	69	26	0.13	1.81	11.3	32	14.2	1.57	0.78
Ligios	890	372	189	32	0.06	1.46	8.00	32	16.0	3.11	2.54
Cuglieri	820	385	37	18	0.09	1.66	6.03	31	10.6	0.23	0.58
Giba	921	379	77	27	0.06	1.39	5.97	36	11.0	0.27	0.68
Mean Scrapie-free	**748**	**366**	**93**	**25**	**0.08**	**1.58**	**7.82**	**33**	**12.9**	**1.29**	**1.14**
Ref mean	500	250	80	15	0.07	1.80	9.00	30	5.0	1.00	2.50

Reference ranges for elements [30,49,78,89].

Table 3 Levels of metals in soils sourced from locations where vCJD cases have emerged in the UK/Sicily [53,54] (Samples collected: October 14—20th 2004)

Sample site	Mn ppm	Ba ppm	Sr ppm	Y ppm	Ag ppm	Cu ppm	U ppm	Th ppm	Al %	Ca %	Mg %
UK											
Longeville,	658	294	128	20	0.2	31	1.90	7.10	6.48	6.08	0.46
Norfolk.	666	168	204	21	0.2	25	1.34	4.34	2.77	16.3	0.30
Lympstone,	744	500	92	13	0.3	21	3.28	8.25	7.59	1.98	0.80
Devon.	44322	1946	95	98	0.1	351	8.55	4.47	10.21	0.21	1.56
Pickenham	1223	291	179	32	0.2	18	1.97	8.8	4.64	4.52	0.68
Queni 1	898	346	97	25	1.4	39	2.84	11.61	11.72	2.81	1.16
Queni 2	1091	368	105	24	1.2	54	2.78	11.14	12.12	2.67	1.17
Queni 3	557	303	97	17	0.2	28	2.01	6.90	7.17	4.19	0.92
Rearsby	859	548	191	38	0.2	21	4.20	19.39	14.06	2.89	2.29
SICILY											
Menfi;											
Bedrock	46	94	173	30	0.1	7	0.79	0.87	0.85	41.49	0.37
Gardens	294	151	117	14	0.3	30	1.19	5.32	4.38	8.51	0.64
Field	279	204	330	21	0.2	18	2.87	9.38	11.02	12.06	1.54
Waste	224	136	183	15	0.1	17	1.24	5.33	3.29	16.22	0.56
SARDINIA											
Arborea (BSE)	418	290	551	27	0.1	26	1.87	6.89	11.38	16.15	1.80
Mean vCJD	**3734**	**403**	**182**	**28**	**0.34**	**49**	**2.63**	**7.84**	**7.69**	**9.72**	**1.02**
VCJD-free											
UK											
4 m South	433	252	49	9	0.2	17	1.34	4.49	3.9	0.40	0.30
Queniborough	387	260	51	12	0.2	20	1.94	6.55	4.38	0.36	0.30
	194	161	44	10	0.1	8	1.33	5.04	2.31	1.45	0.11
Bodney	868	245	47	14	0.2	16	1.62	5.32	4.48	0.36	0.19
Mean vCJD-free	**470**	**229**	**48**	**11**	**0.17**	**15**	**1.56**	**5.34**	**3.76**	**0.64**	**0.22**
Ref mean	500	250	80	15	0.07	30	1.80	9.0	5.0	1.00	2.50

Reference ranges: [30,49,78,89]. Key to sample site abbreviations: Queni 1 = SW on Queniborough Ordnance factory site. Queni 2 = Main drain outfall of Ordnance factory site. Queni 3 = Ex shell fill factory site at Queniborough.

the European TSE cluster ecosystems involved in this study, a near identical profile of elevations were recorded in relation to the analytical studies that were previously undertaken in North American TSE clusters — where Sr, Ba and Ag were increased 2.75×/2.1×/2.6×, respectively (see Table 1) in Europe/Japan and were likewise increased 2.76×, 2.2×, 2.24×, respectively, in North America [27].

The levels of the metals recorded in this study yielded consistent readings across the various TSE clusters that were analysed. There were some exceptions to this rule which involved one or two elements — such as the excessively elevated level of Mn in the Lympstone vCJD cluster, which inflated the overall mean reading for Mn in vCJD clusters to such an extent, that it does not accurately represent the levels of Mn in vCJD clusters.

Water samples were also analysed as part of this study. Whilst significant levels of Ba and Sr were consistently detected in the samples drawn from TSE cluster ecosystems, no unusual observations were provided by this data which warranted their publication.

Discussion; Part 1: The results

The results of this study provided additional evidence that these very rare high incidence foci of TSE have a tendency to emerge in areas which adjoin military facilities — where munitions have been manufactured, tested, incinerated, stored or dumped in the past [3,27] (see Fig. 2). The close proximity of this unique type of facility to these un-

Table 4 Levels of metals in soils sourced from CJD affected villages in rural Calabria [52] (Samples collected: November 1st–7th 2004)

Sample site	Mn ppm	Ba ppm	Sr ppm	Y ppm	Ag ppm	Cu ppm	U ppm	Th ppm	Al %	Ca %	Mg %
New Village	720	647	272	19	0.2	25	1.79	10.2	14.4	6.38	1.39
(20xCJD)	480	752	268	13	0.1	11	1.40	8.10	13.2	5.66	0.87
	697	642	294	19	0.2	22	2.02	10.7	13.9	5.66	1.32
Old Village	3065	435	209	53	0.4	850	2.87	8.83	11.8	2.70	3.21
	1115	1018	198	22	0.3	76	3.57	27.3	20.2	1.24	2.77
	650	600	176	23	0.3	45	3.11	18.1	17.7	1.26	2.75
Village Cit	433	669	189	11	0.2	36	4.67	22.1	16.7	0.35	1.02
Village M	1022	513	144	36	0.7	91	5.32	16.2	17.7	1.18	2.18
Village Po	581	714	168	22	0.3	31	5.26	12.9	16.3	1.09	1.08
	495	678	119	17	0.2	35	3.83	15.1	15.1	0.61	0.67
Village Fo	449	350	476	17	0.1	20	4.37	4.70	16.5	1.46	0.72
Mean CJD	**882**	**638**	**228**	**23**	**0.27**	**113**	**3.47**	**14.0**	**15.8**	**2.51**	**1.63**
Ref mean	500	250	80	15	0.07	30	1.80	9.0	5.0	1.00	2.50

Reference ranges [30,49,78,89].

Table 5 Levels of metals in soils sourced from familial/sporadic CJD cluster (Fuji River Basin) and CJD-free area in Japan [50,51] (Samples collected; April 2002)

Sample location	Sr, ppm	Ba, ppm	Mn, %	Cu, ppm
CJD Cluster (Fuji valley)				
1	206	425	0.12	49
2	281	234	0.16	21
3a	326	252	0.10	51
3b	205	378	0.08	38
4a (peach orchard)	126	465	0.05	59
4b	185	358	0.11	59
5	167	437	0.17	46
Mean CJD	**213**	**364**	**0.11**	**46**
CJD-free (Chiba)				
A	85	304	0.17	78
B	80	236	0.19	109
C	61	218	0.13	166
Mean CJD-free	**75**	**252**	**0.16**	**117**
Ref mean	80	250	500	30

Reference ranges; [30].

ique high incidence hotspots of TSE suggests that munitions provide the most likely source of metal microcrystal pollutants within these TSE cluster zones.

vCJD clustering in the UK

The majority of the 145 cases of vCJD [54] recorded to date have involved people who were raised or spent time in the rural/coastal communities of the UK. And since beef is consumed equally between town and country folk alike, the hypothesis which proposes that beef consumption is responsible for the origins of vCJD is not supported by the predominantly rural upbringing of the vCJD victims. Furthermore, several CJD epidemiologists have made reference to data which demonstrates that a larger proportion of CJD victims have pursued professions or hobbies that are practised outdoors in rural

Table 6 Levels of metals in pasture tissues sourced from scrapie cluster and scrapie-free regions across Sardinia/Sicily [48,53] (Sampling dates; October 24th–November 3rd 2002)

Farm location	Mn, ppm	Ba, ppm	Sr, ppm	Ag, ppm	Cu, ppm	Fe, ppm	Al, ppm	Mg, ppm
Sicily								
S Lucia de Mela	75.6	25.0	54.7	0.69	8.3	602	460.0	2024
Sardinia								
Columbu	816.4	57.22	36.67	2.240	21.37	895	749.0	1782
Bussu G	146.1	31.23	50.64	1.007	17.17	550	313.6	4696
Bussu C.	221.6	68.12	83.00	1.142	22.96	326	184.6	3946
Marchi D.	140.8	53.63	66.94	0.619	14.57	572	441.8	4119
Mareo N.	212.0	59.58	61.56	0.202	18.12	606	476.8	4197
Musei	372.7	31.04	41.16	0.588	20.28	237	172.1	3706
Assimini W.	168.9	124.00	111.9	0.238	22.32	3398	3221	4564
Silanus Birori	151.0	57.83	67.26	0.532	20.44	620	473.8	3372
Ozieri N.	134.0	46.01	25.84	0.254	13.32	425	228.2	2620
Arborea (BSE)	39.0	13.89	87.02	0.410	15.18	636	572.6	6615
Mean scrapie	**252.3**	**51.59**	**69.77**	**0.720**	**17.63**	**806**	**663.0**	**3785**
TSE-free (Sardinia)								
Fertilia West	81.0	10.12	56.64	0.381	21.73	133	115.9	2344
Fertilia	114.5	11.31	10.08	0.172	22.71	817	664.7	1291
Ligios	110.1	23.64	27.56	0.259	30.20	1975	1706	3230
Giba	58.1	21.37	18.01	0.440	25.11	482	330.5	3644
Cuglieri	32.9	13.07	31.16	0.227	21.98	310	252.3	2611
Narbolia	110.4	26.26	33.28	0.279	13.31	861	703.0	3012
Mean scrapie-free	**84.5**	**17.62**	**29.45**	**0.293**	**22.50**	**763**	**628.7**	**3909**
Ref mean	50.0	10.00	20.00	0.050	20.00	200	200	5000

Reference ranges for elements [30,49,78,89].

environments; e.g., farming, forestry, kennel workers, horticulture, stable workers, coastguards, military, horse riding, fishing, exercising pets, shooting, cross country running/outdoor sports.

The CJD Surveillance Unit at Edinburgh had identified nine geographical locations in the UK where two or more spatially associated cases of vCJD had co-emerged [54], but their investigations could not identify any common causal association between these regions and their relationship to beef consumption.

In respect of the metal microcrystal theory, the small English village of Queniborough, and its surrounding area, is a classic example of this association between TSE clustering in rural areas [54] and their close proximity to munition sites (see Fig. 3). Queniborough has hosted the most intensive cluster of vCJD in the world, since six cases of this very rare disease have purportedly been connected to this region. All of these victims had grown up/played around and consumed wild game/fish/fruit grown around the lands of the former 130 acre ''Queniborough Ordnance Depot'' [55] (see Fig. 4) — a facility where processing and storage of chemical weapons, triggers and detonators involving various persistent types of chemical such as phosgene, mustard gas and other 'undisclosed' types of munition [55], had taken place up until the 1960s. During world war two, these munitions had actually been stored along the verges of the lanes around Queniborough in an attempt to avoid the possibility of being hit by the German bombing sorties [55] (see Fig. 5).

The secondary 'acoustic shock' prerequisite of the TSE environmental origin theory is also well evident in Queniborough, since like many English villages, the Royal airforce used the church spire as a marker for their low fly practice jets throughout the 1980s — whereby the shock waves from the overflights had putatively activated the latent piezoelectric potential of any metal-protein crystals carried in the brains of contaminated individuals. This phenomena may explain why vicars — as an occupational group — are blighted by the highest incidence rate of CJD — 7 cases per million [7].

Ref.	Location	Date	TSE type	Munitions connection	Sonic Source
	USA				
[92]	Tucson, AZ.	1978	CJD cluster	Missile factory workers	Workshop tests
[93]	Fort Collins, CO.	1968	CWD cluster in wild/ captive deer	Missile silos, Rocky Flats nuclear munitions factory leak, munition incineration at; Lyons cement kiln and 11 million galls of nerve agent at Rocky Mountain Arsenal	Quarry explosions, Rifle shooting, LF jets, Front range tectonic fault line.
[94]	Mt Horeb, WI	2000	CWD cluster in wild deer	Clean up/incineration of munitions at Badger Ammunition Plant in 1999 [97], Hercules flight path	Explosions for new road, Rifle shooting, Quake epicentre, LF jets
[95]	Kimball, NE.	2000	CWD cluster in wild deer	Incineration of Badger Munitions at Kimball incinerator in 1999 [97], Missile silos.	LF jets, deerhunter rifle shooting.
[94]	White Sands Missile Range, NM.	2000	CWD cluster in wild deer	Missile and bomb testing range.	Missile explosions.
[98]	Mission, TX.	1960s	Scrapie cluster	Former military airbase (WW2). Bomb storage.	Under former take off flight path.
[27]	Garden State, NJ	1990s	sCJD cluster	Fort Dix military Camp, MacGuire airbase.	LF jets, Gun and shell explosions
[27]	Mabton, WA	2004	1st US BSE	Hanford Nuclear weapons Plant, Yakima Military training camp, Othello airbase.	LF jets, Shell explosions.
[27]	Spokane, WA.	2004	1st US vCJD	Hanford Nuclear weapons Plant, Yakima Military training camp, Othello airbase.	LF jets, Shell explosions
	CANADA				
[27]	Nameo, AL	2001	1st Canadian CWD captive deer	Nameo Military airbase	Under take off flight path
[27]	Leduc, AL	2003	1st US BSE cow reared here	Leduc International Airport – mainly civilian	Under take off flight path
[27]	Tulliby Lake, AL	2003	1st Canadian BSE	Cold Lake Airbase and air weapons / cruise missile test range.	Under LF jet practise circuit/ Hercules flight path
[27]	Hillmond, SA	2002	CWD cluster in farmed elk	Fall out from Cold Lake air weapons test range.	Under Lloydminster airport take off path / Hercules

Figure 2 Key TSE clusters around the world and their spatial-temporal association with locations where military munitions have been manufactured, tested, stored, incinerated, dumped, etc. [92,93,96,98–103].

Interestingly, Winston Churchill had ordered the mass manufacture and stockpiling of considerable tonnages of chemical weapons that were to be used as a last resort to defeat the Germans [56] if the need had arisen. Fortunately, they were never used. These weapons were manufactured at Queniborough, Randle and Rock Savage in Cheshire, Springfields in Lancashire and dispersed to munition stores (usually sited in woodland) close to airbases all along the east coast of the UK from Lossiemouth to Kent [56], as well as in North Wales.

					Flight path. Gas well pumping.
[27]	Manitou, SA	2002	CWD cluster in wild deer	Camp wainwright tank shelling range. Detonation / incineration of waste munitions , chemical munitions [99]	Tank shelling, Manitou rifle shooting range.
[27]	Between Lloydminster / Saskatoon.SA.	2002	1st vCJD	Fall out from Camp Wainwright / Cold Lake air weapons range	LF jets, Munition explosions.
	ICELAND				
[18] [19]	Akureyri / NE/ SW areas of Iceland	1950s 1970s	Scrapie clusters	Chemical / conventional munitions dumped in seas - contaminated fish meal protein fed to sheep.	Volcanic / tectonic / thunder shock waves.
	UK.				
[3]	Burnham-on-Sea, Somerset	2000	vCJD cases	Puriton Ordnance Factory, Weston Aerial test range, WW2 airbase.	LF military jets
[3]	Armthorpe, nr Doncaster	2000	vCJD cluster	RAF Finningley	LF military jets [101] flight path, Concorde visits
[54]	Queniborough, leicestershire	1996	vCJD cluster	Queniborough ordnance depot [55], WW2 Bomber crash [100]	LF military jets, Kegworth International airport flightpath, Concorde visits
[3] [102]	Villages north of Tenby, South Wales.	2000 1970s	vCJD cluster sCJD cluster	Castlemartin/Penally/ Manorbier/ Pendine / Pembrey tank shelling /aerial bomb test ranges	LF military jets
[3]	SW Lancashire	1999	vCJD cases	Chorley Ordnance Factory / munition incinerator [63]	LF military jets
[3]	Sunderland area	1998	vCJD cases	Cokeworks munition incinerator.[63]	?
[3]	Lympstone, Devon	2000	vCJD cases	Lympstone marine camp/ Woodbury common practise range,	LF aircraft flight path (Exeter airport), Concorde visits.
[3]	Eastleigh, Southampton.	1998	vCJD cases	Eastleigh works munition factory.Worthy Down test ranges	LF aircraft flightpath, (Southampton airport).
[3]	E Chinnock / Stoke, Somerset	1992	sCJD cluster	Yeovilton Naval airbase	LF military jets
[7]	Villages west of Ashford, Kent.	1996	vCJD cluster	Woodlands used as chemical / conventional bomb depots in WW2. WW2 Bomber /USAF airbases at Headcorn / High Halden. WW2 Bomb Alley [65]	LF military jets, Flightpaths into Heathrow airport and local Headcorn airport [66]
[102]	Villages north	1975	sCJD cluster	Orfordness nuclear /	LF military jet
	of Woodbridge, Suffolk.			conventional bomb factories. Bomber airbase and crash landing in Parham village	flightpath
	Weston Longeville	1998	vCJD case	W Longeville US bomber base / bomber crash site [67] WW2 munition stores	LF military jets

Figure 2 (*continued*)

	Cheviot hills, Romney Brecon hills Berks Downs	Long term	Scrapie cluster regions	Otterburn test range Lydd/Hythe test ranges Epynt artillery range Lambourne ranges	explosions
	FAR EAST				
[96]	Guam	2002	CJD case	WW2 chemical munitions buried in victim's land	LF military jets, tropical storms, earthquake tectonic fault lines
[74]	Highlands of New Guinea	1950s	Kuru cluster	WW2 US Bomber crashes / exploding bombs	Bomb explosions, thunderstorm belt, earthquake tectonic fault line
[3]	Obhiro, Hokkaido, Japan	1950s	Scrapie cluster	WW2 army weapons test range	?
[50]	Fuji valley, Japan	1950s	s/familial CJD cluster	Munitions / film factories, aluminum alloy factories,	Volcanic / earthquake belt
	ITALY				
[103]	Parma region, Italy	1975	sCJD cluster	Munitions factory	?
[53]	Ragusa, Sicily	2000	BSE cases	Comiso USAF airbase. Nuclear cruise missile base [76]	LF jet flight path
[53]	Trapani , Sicily	1998	BSE cases	Trapani Bergi NATO airbase [76]	LF jet / stealth jet flight path
[53]	Menfi , Sicily	2001	vCJD case	Sciacca WW2 Bomber Airbase. Intense bombing.	LF jets / Quarry explosions.
[52]	Aspromonte, Calabria	1990	S/familial CJD clusters	Ordnance / nuclear waste dumping. Explosions.	LF jets, Explosions, Earthquake tectonic fault line
[48]	Barbagia Monte, Sardinia.	1995	Scrapie clusters	Ordnance / toxic waste dumping	LF jets, Quarry explosions
[48]	Assemini, Sardinia	1999	Scrapie cluster	Decimmannu NATO airbase	LF military and civilian jet flight paths
[53]	Arborea, Sardinia	2001	1st BSE case	Capo de Frasco air weapons test range	LF jet practice circuit / explosions

Figure 2 (*continued*)

After WW2, Canisters containing approximately 17,000 tons of the organo phosphate sarin/tabun nerve gases, 14,000 tons of phosgene, 120,000 tons of mustard gas, as well as other waste munitions (white phosphorus, etc.) and propellants (nitroglycerine) were removed from Queniborough and other ordnance/storage depots and were shipped out and dumped in fault lines (Beaufort Dyke, etc.) under the Irish and North Seas [57–60] (see Fig. 6) – areas which have been intensively trawled for the provision of fish protein for both human and livestock consumption in the UK.

The gradual corrosion of these canisters by the sea water or mechanical damage due to the laying of cables on the seabed has lead to reports of munitions leaking into the open seas over recent years and fishermen suffering acute intoxications as a result – indicating the start of a wide scale contamination of the local marine food chain [58,59]. Furthermore, corroding canisters containing white phosphorous/mustard gas munitions have been washed up on Irish and UK beaches since the late 1980s [59]. (NB. Phosphorus represents one of the nucleating agents that can seed metal-protein crystals in biological tissues).

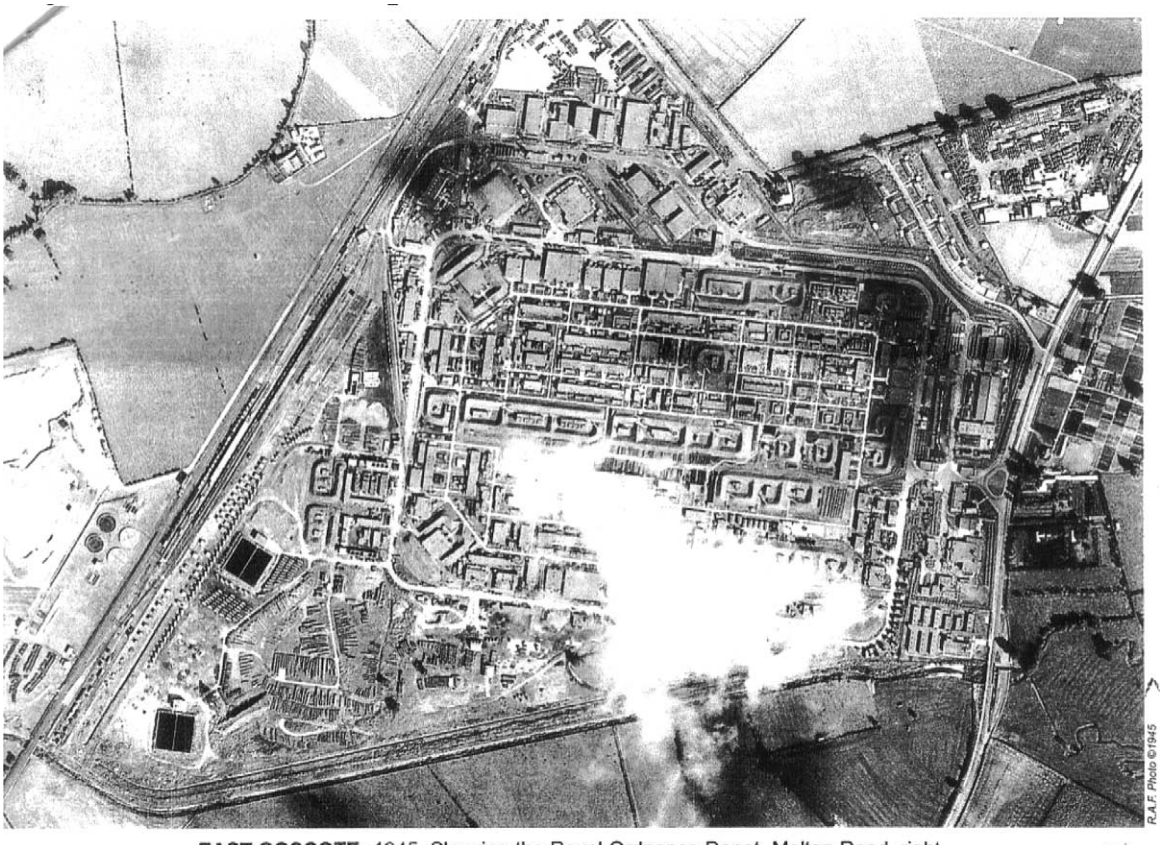
But UK government analyses of sea bed sediment and the 'edible' portion of fish [60] has been unable to identify the parent compounds of the particular types of chemical munitions that were dumped in these seas. It should however be noted that this study failed to analyse the samples for the specific metabolites that are known to degrade via alkaline hydrolysis from these types of chemical ordnance [60]. Furthermore, thousands of tons of the 'unedible' fraction of fish (e.g., the bones and various organs which

Figure 3 The location of cases of vCJD connected to Queniborough and their spatial relationship to the former Queniborough Ordnance Factory [55]. + = location of vCJD case ■ = former site of Queniborough Ordnance factory.

would have bioconcentrated any metal pollutants from surrounding waters, etc.) caught in these waters were directed into UK livestock feeds as a source of protein during the BSE era [61], particularly after the meat and bone meal source of protein was ironically banned in 1988 [14]. But the use of fish meal as a feedingstuff has been considerably restricted since the mid 1990s for conservation reasons, and this drop in usage correlates with the considerable drop in rates of BSE incidence during this period [14].

The intensive feeding of fishmeal protein to the Icelandic sheep population during the winter months, could also offer a plausible explanation for the highest global incidence rate of scrapie TSE occurring in the Icelandic sheepflock [18,19]. The same use of fishmeal feed applies to the Mediterranean sheep flocks affected with scrapie [48]. Long term dumping of both chemical/conventional munitions and military radioactive waste on the seabeds around the coasts of Iceland and Southern Italy is well recognised [57,58]. Likewise, other animal species fed substantial amounts of fishmeal derived from the North Atlantic seas, such as mink, have also endured outbreaks of TSEs [1].

In respect of human CJD incidence, fish, as opposed to beef, may represent a more pertinent



EAST GOSCOTE: 1945. Showing the Royal Ordnance Depot. Melton Road, right.

Figure 4 WW2 aerial photo of the Queniborough Ordnance Depot [55].

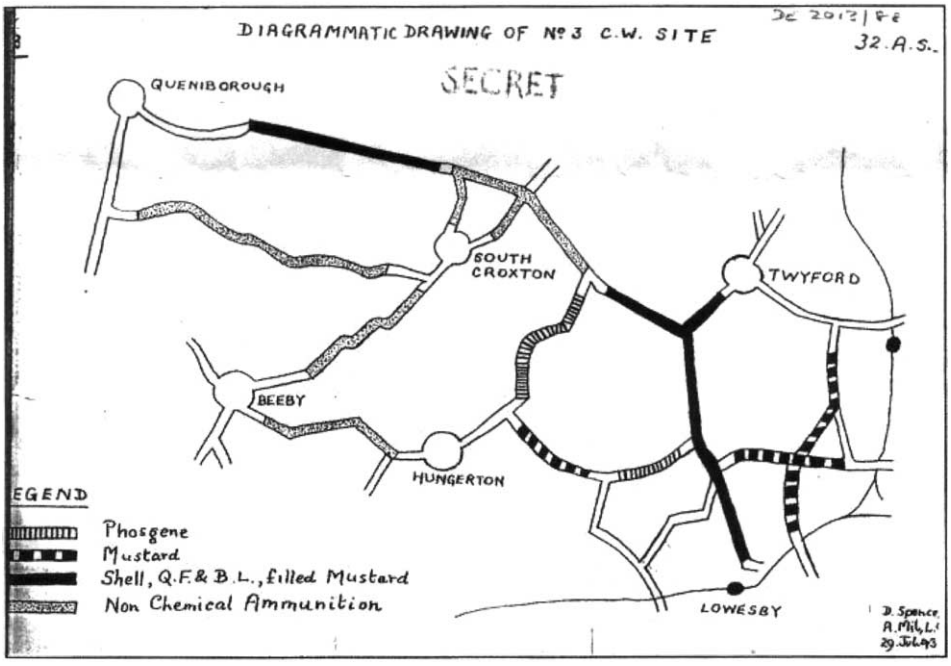


Figure 5 WW2 Map from Leicester County Council depicting sites of chemical weapon storage around vCJD cluster at Queniborough [55].

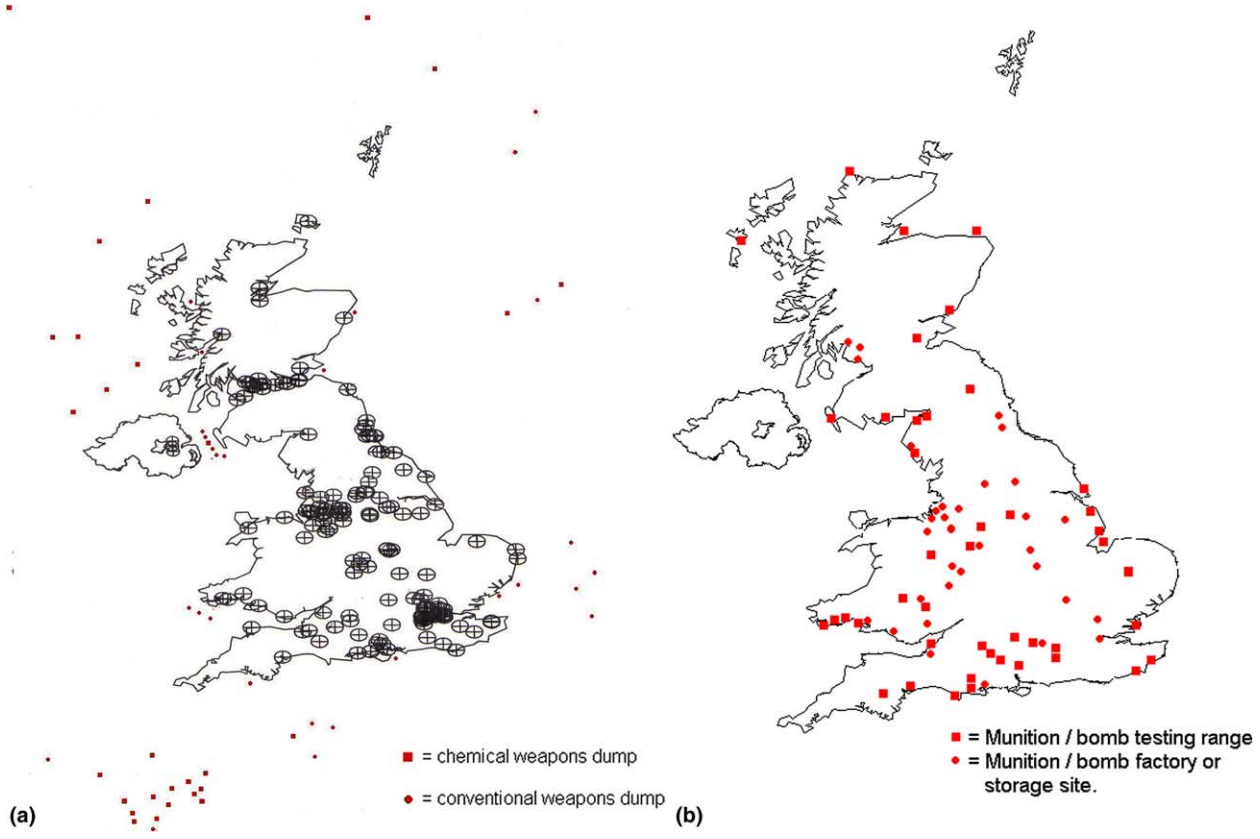


Figure 6 (a) Distribution of cases of vCJD in the Uk [54] in relation to the sites of marine dumping of chemical and conventional munitions since WW2 [58]. (b) Distribution of Munition factories and military test ranges across the UK since WW2.

candidate prerequisite in the multifactorial aetiology of BSE/vCJD (see Fig. 7); perhaps explaining why the human residents of the coastal areas of Northern Britain — notorious for their customary high level consumption of fish and chips — exhibit the highest rates of vCJD [54] (see Fig. 6). It is interesting that all cows that were home reared upon fully converted organic farms have remained totally BSE-free [16], and organic farming standards had debarred the use of fishmeal as feed during the 1980s.

It should also be noted that more recent manufactured sources of unexploded ordnance/ordnance contaminated soils from a variety of ordnance depots across the UK were processed into sludges for incineration [62,63]. Perhaps more disturbingly, they were processed into the 'secondary liquid fuels' that were widely used for controlled incineration in cement kilns, cokeworks, etc. [62,63] across the UK — a practice that accelerated prior to privatisation of the UK Ordnance factories in 1987. Furthermore, during the early/mid 1980s, there was considerable research into the development and incorporation of the resulting fly ash from these kilns — known as aragonite — into all types of animal concentrated feeds [64]. The aragonite was valued as an additive since it contained beneficial minerals such as calcium [64], although any animals that consumed this product were unwittingly exposed to a cocktail of other metal excesses; such as Sr, Ba, Mn, etc., that were not so desirable from a nutritional perspective. The feeding of Aragonite provides another plausible route through which metal microcrystals could have entered the farm food chain and 'seeded' the UK epidemic of BSE (see Fig. 7). Likewise, its use as a feed additive throughout other European countries — albeit at considerably reduced rates of inclusion — could explain the lower incidences of BSE throughout other European countries [14].

Other hotspots of vCJD have emerged in rural districts of the UK, such as in a group of villages north of Tenby (Wales), at Lympstone (Devon), in villages west of Ashford (Kent), at Armthorpe (west of Doncaster), Burnham on Sea (Somerset), SW Lancashire and Sunderland (see Fig. 6 [54]). These locations are all sited adjoining facilities that have been associated with manufacturing/testing munitions in some way [3] — at Castlemartin/Penally/Pendine Sands test ranges, Lympstone marine barracks/Woodbury test ranges, WW2 bomb alley/WW2 woodland munition stores, RAF Finningley, Ordnance factory at Puriton/Weston supermare aerial test range, Ordnance factory at Chorley, Lambton Ordnance incinerator in Sunderland, respectively (see Fig. 2).

Five cases of vCJD that emerged in the early stages of the epidemic involved the rural residents of (or frequent visitors to) a group of adjoining villages west of Ashford in Kent [7] — High Halden, Bethersden, Egerton, Sissinghurst, etc. These villages had hosted a plethora of military airbases in world war two — such as Lashenden and High Halden — [65] which were served by substantial munition/bomb stores located in the small woodlands of that region (e.g., Foxen Woods, Smarden woods, Frith Woods, etc. [65]) and the author's investigation has revealed that it was customary of the local population to consume wild game (pheasant, rabbit, etc.) and berries/nuts that had thrived upon these potentially contaminated woodland ecosystems.

The kent cluster was also located along the infamous 'Bomb Alley' [65]; the London–German corridor which experienced a persistent stream of military air traffic and explosive encounters during world war two.

The sonic prerequisite is also well evident in this region and is substantiated by a publication into noise mitigation measures taken around Headcorn aerodrome [66], where the villages of High Halden and Bethersden are considered to be the most affected. Furthermore, the sonic problem is compounded by the fact that this region lies directly beneath one of the main flight paths into London Heathrow's International airport [66].

Other outbreaks of vCJD were investigated. One was centred in a village in rural north Norfolk adjoining one of many WW2 bomber airbases in this region of the UK. A US lincoln bomber had actually crashed into the village at the end of the runway, having failed to take off properly due to the weight

Phosphorous Use as systemic organophosphorus insecticides for warble flies, etc, on cows. Also contaminated fishmeal protein (used for cattle and poultry feeds) sourced from UK waters as a result of munition dumping after WW2.

Silver Use as biocide in water supplies on poultry and dairy farms, thereby entering the farm food chain via water, poultry manure (in feed and fertiliser), MBM feed, etc. Use as detonator in munitions contaminating fishmeal, etc.

Strontium 90 Deposited over NW Europe as a result of the plume of Chernobyl nuclear accident – concentrated into alfalfa / clover,etc, pastures. Bonded onto ferritin protein and contaminated farm food chain. Bioconcentrated into the bone meal fraction of feed and recycled back into the bovine.

Barium Contaminated fishmeal as a result of munition dumping and use of Ba in oil drilling mud in the seas around the UK.

Figure 7 Possible pollutant sources of metal microcrystal nucleator that could have 'seeded' the growth of the metal-protein crystals that caused the Uk's BSE epidemic (exacerbated by overuse of vitamin D3 feed additive).

of bombs on board [67]. These had detonated during the crash landing.

Interestingly, several intensive turkey farms had been established during the 1980s on the sites of these former airbases. They were developed across the whole of Norfolk county in the Eastern England. Whilst metal microcrystals entered the foodchain of turkeys due to the customary feeding of fishmeal protein to all poultry during the BSE era, the turkeys in these Norfolk based units were exposed via other routes since they had direct access to peck at the bare earth/concrete runways for grubs and the grit for their gizzards. So any residual munition or other pollutants left over from the military occupation could have entered the farm/human food chain via these Turkey enterprises.

One route through which these munitions could have bioconcentrated further up the farm foodchain in areas like Norfolk, involves the intensive use of this turkey/poultry manure as a fertiliser; where it was liberally spread across the grassland of adjoining dairy farms and subsequently taken up into the grazing cow. Combined with the fact that this region of Norfolk is home to many operational military airbases — whose low fly jets must generate intensive levels of sonic shockbursts — It is perhaps no surprise that the highest rates of BSE in cattle have been confined to this 'turkey belt' region of Norfolk throughout the BSE era [14].

Some of the regions of the UK noted for their higher incidence of scrapie TSE in the local sheep flocks — e.g., the Cheviot Hills, the Berkshire Downs, the Brecon Beacons, Romney Marsh — are also regions that have hosted the most substantial military shooting ranges in the UK for many decades; e.g., Otterburn, Lambourne Downs, Epynt, Lydd/Hythe ranges, respectively.

CJD/scrapie clustering in Calabria/Sardinia (see Fig. 8)

Observations resulting from the Mediterranean facet of this study, highlighted the unsanctioned activities of the eco-mafia which involved the burial/incineration of unexploded ordnance and other toxic wastes within the Aspromonte and Barbagia mountain regions of Calabria and Sardinia [68,69]— regions where both the local human/farm animal populations have recently demonstrated an increased incidence of TSEs [48,52,53], Alzheimer's disease, brain tumours, etc., since the 1990s.

Collusion between the mafia and the military was first established after world war two [70], when the allied armies had eagerly offloaded their stockpiles of unused ordnance via many of the mafia clans operating in Calabria and Sicily at that time. Today, the situation is little different, where a whole network of industrial bodies are covertly paying the eco-mafia groups a 'cut price' rate to dispose of a wide array of toxic chemical and radioactive wastes. These are invariably transported to one of the many illicit 'fly tips' in the South of Italy where they are incinerated, dumped directly into mountain ravines or incorporated into building blocks for houses [68,69]. Calabria plays host to over 400 of these unofficial toxic dumps [69].

One such contaminated region had implicated the entire Italian—Greek population of a tiny Calabrian mountain village (referred to as *Old Village* in Table 4) who had to be rehoused by the government in a brand new village (referred to as *New Village* in Table 4) down by the coast; where 20 cases of CJD later emerged within 142,000 sq metres of settlement area over a five year period [52].

The cases of BSE, scrapie and variant CJD investigated in Sicily and Sardinia [48,52,53] involved locations that were sited in areas where celestine and other strontium/barium rich bedrocks [71] have been increasingly quarried for the production of cement and gypsum used in the construction of roads and hotels, etc., to meet the demands of the fast expanding tourist industry. The author's analyses confirmed elevations of Ba and Sr in the local ecosystems (see Tables 2—6). NB; Gypsum quarrying/processing is also well evident in the vCJD cluster around Queniborough in the UK, and the CWD cluster around Fort Collins in Colorado [1].

The TSE outbreak foci also lie very close to the incoming/outgoing flightpaths serving the large Naval and NATO airbases at Sigonella, Cameiso and Trampani Bergi on Sicily, and at Decimomannu airbase on Sardinia — and beneath the low fly practise zones of the Decimomannu airfleet at the Barbagia monte and the Capo Della Frasca aerial bomb test range — the latter being 4 km distance from Sardinia's single outbreak of BSE at Arborea [53] (see Fig. 8); thereby providing the source of sonic shock prerequisite required for these TSE oubreaks.

Sicily vCJD case

The case of vCJD in the small village of Menfi in Sicily was sited in an area of open cast Sr celestine quarries and cement production, and next to the former world war two bomber airbase at Sciacca. In the first days of July 1943, Sciacca and Menfi endured a sustained aerial bombardment of ordnance during the allied invasion of Sicily in WW2 [72]. The

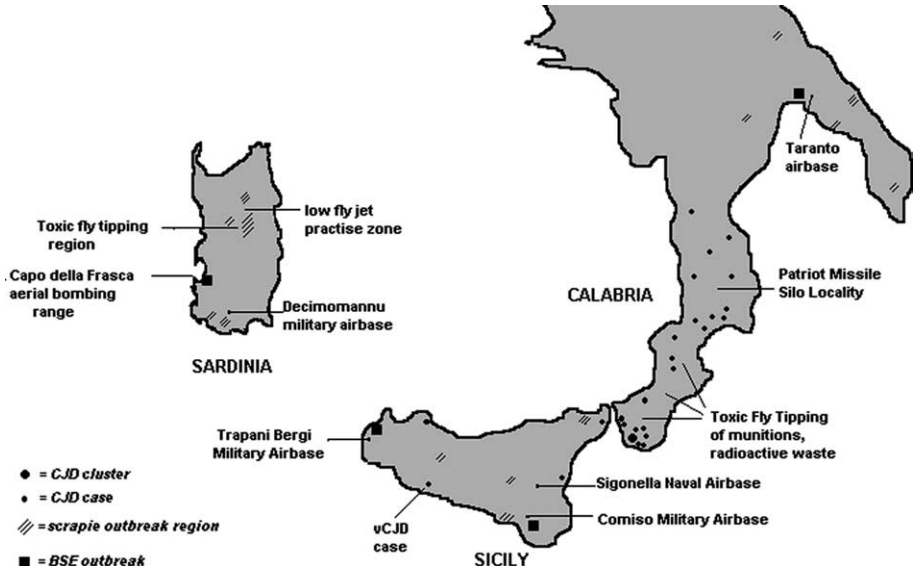


Figure 8 The Militarisation of the Mediterranean and the distribution of known TSE outbreaks since 1995 (source references [46,49,50,79]).

US Chemical Corps, who fired white phosphorous and phosgene shells [73] were also involved in this invasion.

The precise correlation between the locations of many TSE clusters all over the world (see Fig. 2) and the locations where world war two bomber aircraft/munitions have exploded provides the subject for a fascinating novel environmental investigation.

Fuji valley sporadic/familial CJD cluster

In Japan, a long term cluster implicating both sporadic and familial cases of CJD has been recorded amongst the small scale, subsistent farmer community who reside along the Fuji river basin since the 1950s [50,51]. Possible sources of the elevated levels of Sr that were observed in this ecosystem (see Table 5), could relate to the emissions from the Mount Fuji volcano. Likewise, another contributory source of metal microcrystal could stem from the intensively industrialised mouth of the Fuji valley, where film, munition and aircraft construction factories have operated since before world war two [26,3]. As the prevailing winds blow in from the southern coastal mouth of the valley, the atmospheric contaminants vented from this industrial area would tend to be carried northwards along the river basin where the incidences of CJD have emerged [50,51].

It is interesting that several of the military bombers that were constructed out of the metals that were forged at this factory, had been shot down by the US warplanes during world war two over the region of the Highlands of New Guinea which represented the territories of the Fore Tribe [74]. The Fore tribesmen had scavenged much of the metal from the downed bombers to forge into tools and cooking pans, etc. They had also accidentally detonated some of the unexploded bombs on these aircraft [74]. Perhaps it is no coincidence that an epidemic of CJD (known as kuru) subsequently emerged amongst the survivors of these explosions during the early 1950s — first discovered when a Western explorer had a chance encounter with this neurologically crippled community [74,38].

Another TSE cluster in Japan involved an excessive high incidence foci of scrapie that emerged in a flock of sheep that had been introduced onto a remote patch of hill land at Obhiro in Hokkaido [26]— an area that had been exclusively occupied by the Japanese military up until the end of WW2 and used as an experimental firing range. Numerous animal experiments were carried out at this site as well, where horses had been deliberately exposed to detonations of various 'undisclosed' munitions [26]. Although this specific cluster area has not been analysed by the author, geochemical sampling of the indigenous Obsidian derived soils around this cluster has demonstrated consistently elevated levels of Ba ranging between 1500 and 4000 ppm [75].

The traditional consumption of nuts amongst CJD cluster populations accentuates the uptake of Ba and Sr into the biosystem

Another interesting observation resulting from the study of the TSE clustering in the Mediterranean areas of Calabria, central Italy and Sicily [48,52,53] (as well as Slovakia [2,76,77]), involves the local custom of nut consumption, especially walnuts, as one of the mainstays of the local population's traditional diet.

Walnuts, and other nuts, are well recognised to concentrate extremely high levels of Ba/Sr/radium [30,78,79] especially when they are cultivated in the high Ba/Sr granitic/celestine/dolomite soil types [71] which exist in the regions where TSEs have become prevalent.

However, providing the presence of these earth metals has not stemmed from man made sources of pollution, their elevation in the local geology and foodchains of these TSE affected populations must represent a geochemical profile that has remained virtually static for thousands of years; thereby failing to explain the sudden emergence of TSE outbreaks since the 1990s. However, the introduction of some hitherto unrecognised novel factor into these cluster environments — which may be interacting synergistically with the already elevated levels of Ba/Sr — might explain the temporal epidemiological perspectives of these TSE outbreaks.

Radioactive metal microcrystals as the trigger of new strain TSEs?

It is a feasible that the introduction of the ionising radiation that was released from the Chernobyl nuclear power station accident in April 1986 [76], was absorbed by the large quantity of natural and/or industrial sources of Ba/Sr present in the topsoils of these regions and subsequently mobilised up the food chain into the mammalian biosystem. For Sr/Ba concentrated materials such as gypsum are well known to absorb and carry significant levels of natural radioactivity. In this respect, any individual who consumes the types of food which carry high concentrations of Ba/Sr — such as walnuts [30,78,79], alfalfa [80] and pine/juniper [27] — in radioactive polluted environments, will naturally carry higher levels of radioactive contamination within their biosystems than the individuals who do not eat these types of food. The observation that the TSE affected populations tend to consume these types of foods [2,27] in environments where radioactive contamination has posed an issue in the past, may therefore prove to be relevant to the aetiology of TSEs.

In this respect, radioactive metal microcrystals that have polluted local ecosystems as a result of leaks from military munition or domestic power or natural sources could initiate the more virulent, aggressive modern strains of TSE, such as vCJD or BSE. The more aggressive, widely distributed and shorter term pathogenesis observed in these new strain TSEs would result from the overall enhancement of deleterious free radical chain reactions within the tissues due to the compounding hyperoxidative effects induced by the radioactive decay. Whereas the non-radioactive metal microcrystals could initiate the less aggressive traditional strains of TSE [2] due to their more 'laid back' pro-oxidant capacity resulting from their piezoelectric-magnetic effects.

In this respect, the upsurge in incidence rates of various types of TSEs across Europe during the 1990s [14,48,52,53], could have resulted from the massive release of radiation from the 1986 Chernobyl eruption which permeated the foodchains and water supplies of the various species that have subsequently developed TSEs. The radiation absorbing metals, such as Ba and Sr, present in those contaminated environments would have acquired an additional free radical generating pathogenic capacity — dependent upon the amount of radiation that they had absorbed.

Furthermore, the area of the Mediterranean which hosts these TSE clusters was also intensively militarised with nuclear weapons during the cold war era, with bases like Comiso in Sicily playing host to the storage of nuclear cruise missiles. The passage and docking of nuclear submarines in the seas around the straits of Messina and the Sardinian/Sicilian naval bases could also present a problem of pollution [81,82] (see Fig. 8).

The putative radioactive nature of the metal microcrystals that are bound up within the TSE diseased brain, may explain why ten times higher levels of manganese has been observed in the CJD diseased brain [23] in relation to brains sourced from those who have died of non-neurological diseases. Whilst this elevation was originally assumed to be the result of contamination by an external environmental source of Mn [2,3] — which could still be the case — it may also reflect the customary upregulation in MnSOD expression and related Mn mobilisation that follows exposures to ionising radiation — where the synthesis of Mn SOD has been switched on to provide the brain tissues with an antioxidant defence against the radicals generated by the radioactive exposure [83].

Furthermore, since the ferritin molecule is therapeutically used as a chelator of strontium 90, plutonium, etc. in victims of radioactive metal poisoning [84], the well recognised incorporation of endogenous ferritin protein into the rogue PrP fibrils [85] within the TSE diseased brain might indicate that the radioactive contaminated biosystem has selected the use of ferritin as a radioactive chelator in its best line of defence against contamination by radionuclides such as strontium 90, etc. — whereupon the formation of these aberrant ferritin-prion protein fibrils in the TSE diseased brain might merely represent the *tombstone* legacy of the body's battle against exposure to these invasive radioactive metals.

Does a radioactive or chemically initiated autosomal dominant type mutation in a previous generation serve as the primary initiator of nvTSEs?

It is possible that some TSEs result from the exposure of previous generations to radioactive metals or chemical mutagens which had initiated a mutation in the PrP or S-proteoglycan cell line. This aetiological explanation addresses one of the key epidemiological flaws that threatens the viability of the munition — TSE theory, which involves the considerable delay period, by as much as five decades, that exists between the emergence of some of the more recent clusters of TSE and the period when these cluster environments were first exposed to the toxic 'fall out' from these munition sources during WW2.

Whilst the classic course of the TSE types of neurodegenerative disease are well recognised to exhibit a pattern of delayed emergence/prolonged 'incubation' period, the excessive delay period between the time of exposure to the munitions during WW2 and the emergence of some of these clusters during the 1990s — which implicated vCJD victims as young as 20 years old — must indicate that the cause and effect of this exposure has spanned one or even two generations.

If the causal association between these WW2 munitions and TSEs is indeed correct, then the protracted delay period of some cluster outbreaks could simply relate to the fact that the toxic chemicals/metals contained in the dumped/discarded WW2 munitions have only recently gained access to the open environment due to man made disturbances — such as earth moving or sea bed operations — or due to the eventual corrosion of the canisters in which the munitions were originally contained.

Alternatively, this delay could be explained by the mutagenic effects of some chemical or radioactive components within the munitions. These had initiated a mutation in a cell line of a previous generation that had been directly exposed to high doses of the mutagen. In this respect, the clinical expression of the mutation has jumped a whole generation before it has manifested itself; whereby one of the parents or grandparents of the TSE victims had acquired the original 'founder' mutation.

The proposal of an environmentally induced mutation is plausible in that many of the metals (e.g., Strontium 90, uranium, barium [79]) and/or other chemical components implicated in the munitions (e.g., the alkylating Organo phosphates, Nitrogen Mustards, phosgene agents) that have been observed in the TSE cluster zones are well known to exert mutagenic effects on cell lines. And these could be impacting the genes that encode for the synthesis of molecules that are centrally involved in the pathogenesis of TSE — like PrPc, or the sulphated proteoglycans/pyrophophatases that regulate biomineralisation.

In this respect, several strains of scrapie and CJD have already been shown to stem from point (amino acid substitutions) and insertion/deletion mutations involving PrPc [1], or from mutations that bring about the synthesis of ''non-sulfated'' forms of a S-Proteoglycan heparan motif [109]. However, the scope of the surveillance work involved in the identification of mutations in TSE pathogenesis has largely only focused on the PrP cell lines to date, and a broader based surveillance programme needs to be launched in order to identify the possibility of other types of mutation — such as a frame shift — that may serve as primary initiators in the origins of the sporadic or new variant forms of TSE that have never been previously associated with a genetic origin. In this respect, mutations may well be identified in the cell lines of the other 'non-PrP' proteins that have been implicated as co-contributors to the TSE disease process.

Furthermore, the possibility that the 'founder' carriers of the mutations identified in the familial strains of TSE were exposed to environmental mutagens also needs to be explored, since chemical/radioactive induced point/insertion/deletion mutations are well known to express themselves as autosomal dominant disorders whenever homozygosity manifests itself within the succeeding generations.

An expansion in this area of TSE research would also help to decode the pathogenic mechanisms operating in TSEs, in that the biochemical/metabolic mode of expression of any mutation identified would highlight the specific point of

disruption in the cellular metabolism of PrPc, S-proteoglycan heparan's or pyrophosphatase that was involved. For example, if the defective cell line encodes for a mutant Cu binding ligand on PrP, that mutation would invoke pathogenicity as a result of its disruption in the successful binding of Cu to PrP. Or where the mutation has encoded for the synthesis of a non-viable S-proteoglycan, then this would induce a misfolded PrP conformation and a dysregulation of the mineralisation process, etc.

Low Cu, Zn, Se in TSE cluster foodchains

The analytical results yielded in this study, as well as the previous N. American study, [27] do not support earlier observations that the levels of Cu and zinc (Zn) are deficient in TSE cluster foodchains [2,3]. In fact, some of the Cu readings were excessively elevated in the TSE clusters analysed (see Tables 2–4). However, it is the loss of copper binding to PrP's metallo binding domains that represents the '*bottom line*' of this pathogenic prerequisite [2], and these latest results do not preclude the possibility of a secondary Cu deficiency – as opposed to a primary environmental Cu deficiency – fulfilling this prerequisite. For instance, the elevated levels of Ag in the TSE cluster ecosystems investigated [27] could bring about a situation where certain Ag species (of ionic radii 93/81/89 pm) are able to competitively displace Cu atoms (ionic radii 91, 87, 74, 71 pm) at Cu's binding domains – since the similarity between the ionic radii of some Ag species and Cu species, explains why Ag demonstrates a greater binding affinity at some Cu ligands than Cu itself [32].

In respect of the sulphur deficiency prerequisite that has been previously observed in TSE ecosystems [27], the results of this study are also supportive. For the elevation of Ba and Sr in the ecosystem will bring about a chelation of free sulphur, thereby depriving the foodchain of available sulphur; which, in turn, will deprive the biosystem of those who are self sufficient upon the local foodchain. This lock up of sulphur will impair the synthesis of viable S-proteoglycan molecules that play a vital role in maintaining the conformational stability of PrPc [44,45], in growth factor signalling [108], in crystal inhibition [91], etc., and, therefore, in preventing the development of TSE.

Exposure to rogue metals like Ag (which will successfully compete against Cu for binding to PrPc) or to Cu chelating chemicals (such as the organo sulphur compounds) will both deprive PrPc of a supply of its correct metal co partners [28,86]. In this respect, a disruption in the binding of Cu onto PrP and a lack of free sulphur required for the synthesis of viable proteoglycans [87,88] will disrupt the cellular function of both PrPc and sulphated proteoglycans respectively; thereby facilitating the successful implantation and seeding with these rogue metal microcrystals.

In respect of the similarities of ionic radii measurements between the foreign replacement metals and the native metal co partners of these proteins, it would be Mn 3+ (72/78 pm), uranium (87/90 pm) and Ag (81/91/93 pm) that possess specific replacement or co-binding affinity for the Cu (71/74/67/91 pm) and Zn (74/88/104 pm) metal domains on PrP [2,22,27], whereas Ba (149/153 pm) and Sr (132/140 pm) would readily bind into the metal centres on S-proteoglycan heparans [88].

Chelation or displacement of 'available' Cu will also lead to an increase in pyrophosphatase activity [89] – an enzyme which is required for hydrolysing the pyrophosphate which exerts an inhibitory mode of regulation of the crystallisation process involved in the mineralisation of bone [90]. So when pyrophosphatase activity is increased, pyrophosphate levels will subsequently decrease and the aberrant hypermineralisation of soft tissues will ensue.

Lack of available Cu will also serve to restrict the viable synthesis of certain antioxidant enzyme groups that neutralise the free radical chain reactions that are generated by oxidative stress [40]. In this respect, it has been suggested that the electrical/magnetic fields (in combination with any radioactive decay) that are putatively generated on the surfaces of the metal-protein crystals within the TSE diseased brain [27], provides the source of pro-oxidant stress that is implicated in propelling the progressive pathogenesis of TSEs [2].

Discussion: Part 2

A Nutritional approach for preventing the aberrant mineralisation of the soft tissues of the CNS. A preventative for TSEs?

It is suggested that various treatments/supplements could be introduced into the specific TSE cluster ecosystems and/or fed to local populations that are at a high risk of contracting TSEs. This would involve straight forward fertilising/supplementing of the soils or foods with the appropriate

Cu/Zn/S mineral/element formulations — combined with the feeding of varieties of foodstuffs which contain adequate levels of these trace minerals, as well as restricting feeding of vitamin D3 additives. This may be all that is required to maintain a balanced supply of precursor minerals for sustaining a healthy cellular turn over of PrPc and S-proteoglycans; thereby maintaining the stability of their molecular conformations, as well as protecting their metallo domains against replacement bindings by the specific types of rogue metal microcrystal that can successfully compete for binding as well as seed the growth of the metal-PrP-ferritin fibril crystals which putatively initiate TSE.

Likewise dietary supplementation or use of foodstuffs containing the various crystal inhibitor compounds — pyrophosphates [90], magnesium sulphates [91], sulphated glucosamine proteoglycans [42,91] — can act as replacements for any metal induced deficiencies of the endogenous crystal inhibitor compounds that are normally synthesised and available within the healthy biosystem. Likewise, the supply of optimum levels of Cu ions into the biosystem will also inhibit the synthesis of pyrophosphatase [89].

The role of natural sulphur as a potential preventative for TSEs is also extremely important; not only because optimum S will ensure the viable synthesis of S-proteoglycans within the biosystem, but also for maintaining adequate levels of free S that can act as a toxic sink for conjugating with any incoming Ba, Sr, Ag pollutants and rendering them less soluble.

Discussion: Part 3; Biochemistry

Elevated exposure to Ba, Sr or P nucleating agents promote a rogue hypermineralisation of the CNS soft tissues. The possible pathogenic pathway involved

Ba/Sr promotes hydrolysis of the pyrophosphates that would normally inhibit the mineralisation process

Ba and Sr are known to promote the activity of pyrophosphatase in the biosystem [89], which will irreversibly hydrolyse pyrophosphates [104]. Since pyrophosphates are found on the bone surfaces where they play a key role in the inhibition of bone mineralisation [105], this could elucidate how an elevated Ba/Sr induced promotion of the hydrolysis of pyrophosphate into its basic phosphate metabolites could effectively promote a rogue run-a-way hypermineralisation of the soft tissues.

The Ba/Sr induced promotion of pyrophosphate hydrolysis would also lead to a marked deficiency in the optimum levels of pyrophosphate that is required for maintaining the stability of the beta sheets in the final tertiary stages of protein folding [106]. This would compromise the folding of proteins, like PrP, in such a way that the resulting conformations of the affected proteins would present as aberrant protease resistant, misfolded isoforms as observed in the brains of those who have died of TSEs [1] and other neurodegenerative disorders.

In this respect, it is interesting that Cu ions have been shown to induce the opposite effect on pyrophosphatase activity in relation to Ba [89], whereby Cu decreases pyrophosphatase turnover, which, in turn, decreases the rate of mineralisation. So once a deficiency of copper binding to PrP and other proteins comes into play (e.g., as a result of the aforementioned pollutant induced Cu chelation or due to the preferential competitive binding of Ag to Cu ligands [2,27]), then the Cu mediated mode of regulating pyrophosphate metabolism could be disrupted, rendering surrounding tissues susceptible to increased mineralisation.

Ba, Sr, Ag chelation of sulphur disrupts the viable formation of S-proteoglycan heparans that normally serve as endogenous inhibitors of mineralisation

The well known ability of Ba, Sr, Ag or Mn to conjugate with free sulphur within the biosystem [30,31,88,89], could also compound the complication of the progressive proliferation of crystal growth; since any sulphur chelating agent would disrupt the viable synthesis of sulphated proteoglycan group of molecules — some of which act as endogenous bio-regulators of hydroxyapatite crystal formation [42,90,91] that represent the integral components of the bone matrix, as well as the aberrant crystalline structures which are deposited in the soft tissues during the pathogenesis of various pathological conditions such as TSEs, Alzheimers disease [107] and rheumatoid arthritis [91].

Loss of S-proteoglycan activity is considered to be contributory to the misfolding of PrPc and the development of TSEs [43—45], as well as being responsible for many of the features of the TSE disease process — such as loss of S-proteoglycan mediated fibroblast growth factor cell signalling [108]. Further evidence in support of this putative pathogenic prerequisite stems from the fact that some strains of sheep scrapie have been solely induced by mutations in the S-proteoglycan cell lines [109], where the abnormal formation of non-sulphated proteoglycans lead to the development of scrapie TSE.

In this respect, it is proposed that the various rogue Ba, Sr, Ag or Mn sulphate complexes may act as viable replacements at the straight sulphur bonding sites on the prion protein [29] and sulphated proteoglycans [88] — for example, by binding up with the disulphide bonds during the critical tertiary folding stages of the prion protein, thereby disrupting the formation of the all important 3D tertiary conformation of PrP [29].

Sr and phosphorus are well recognised for their ability to act as nucleators in the mineralisation process

Moderate dose rates of Sr compounds are recognised to act as nucleating agents which can initiate the mineralisation process [110,111]. This is well illustrated by the use of the Sr ranelate compound in the successful treatment of osteoporosis [111]. Likewise, various organo-phosphate (OP) compounds have also been shown to promote mineralisation [90], but, much like Sr, the promoting effect will only operate when moderate dose rates have been employed. On the other hand, higher doses of both Sr and phosphates have caused a total reversal in the mineralisation effect [90]. This interesting phenomena indicates that, in the contexts of TSE, high doses of these nucleating agents will invoke a curative effect that could actually reverse the TSE disease process, whereas the more moderate doses that are likely to be encountered during chronic environmental intoxications with Sr or OP compounds would invoke a causative effect.

The curious '*Jekyll and Hyde*' capacity tied up in the dose differential of both OP and Sr nucleators — and how this determines their opposing effects on the mineralisation process — could be relevant to the model of metal intoxication proposed in this paper, and to an earlier toxicological model proposed for the cause of BSE; where compulsory treatment of UK cattle with a specific type of systemic acting organo dithiophosphate insecticide (empowered under the ''Warble Fly Order England and Wales 1982'' [7,112]) represented the primary causal prerequisite that was responsible for initiating the UK's BSE epidemic [113,114] (see Fig. 7).

The fact that the UK suffered approx 250,000 cases of BSE whilst the other BSE affected countries in Europe suffered considerably smaller incidence rates [14], could be explained by the fact that the UK was unique in using up to fourfold higher doses of these systemic OPs to control the warble fly in relation to the application rates of the other European countries [114]. Warble fly free countries in the southern hemisphere, like New Zealand and Australia [115], have remained BSE-free to date [14].

The marked difference in the BSE incidence rates between the UK and other European countries [14] may relate to the different frequencies and dose rates of the OP treatments employed for warble control in the different countries, and how this relates to the potential of the phosphate to promote or reverse the mineralisation process. Interestingly, Whatley et al. [116,7] exposed neuroblastoma cells to various doses of the organo-dithiophosphate warblecide. The cells exposed to the highest doses of OP used — which considerably exceeded the 'in vivo' concentrations of OP found in nerve cells after a 20 mg/kg bodyweight dose had been applied to UK cattle in the field — caused the abnormal protease resistant prion protein to refold back into its normal cellular form, indicating a potential curative effect (e.g., reverse in the mineralisation effect?) following exposure to high doses of OP. However, high doses of OPs could never realistically serve as an 'in vivo' cure of TSEs, since the OP's anti-cholinesterase effects would prove lethal at these high dose rates.

It should be noted that various papers in the literature discount a role of OP warblecides in the origins of BSE [117], but this critique has been based on the erroneous misinformation that the use of OP warblecides had ceased by 1982 in the UK. On the basis of this flawed premise, the authors argue that OP warblecides were never applied to the cows that developed BSE, since the affected cows had largely all been born *after* 1982. Ironically, the opposite is correct. Under the ''Warble Fly Order England and Wales 1982'' [7,112], the use of the OP warble fly dithiophosphate insecticides *first* became compulsory as a twice annual application in *1982* in the UK.

In this respect, the systemic organo dithiophosphate insecticides — which had to be poured along the backlines (spines) of the cows in an oil based formulation — could have provided a phosphorus nucleator that was directly delivered into the CNS of the treated cow, whereupon it seeded the progressive hypermineralisation of the bovine brain and spinal cord; manifesting as the phosphorus-prion protein-ferritin crystals that putatively caused BSE. It is possible that a combination of phosphate nucleators from various sources (e.g., from fishmeal contaminants and OP insecticides) were contributory in the 'seeding' of BSE.

Furthermore, the two free sulphur atoms on this dithiophosphate molecule [118] could have enabled a rogue replacement binding of this pollutant molecule onto the disulphide bonds on prion protein [29],or, alternatively, onto the sulphur binding

sites on the S-proteoglycan heparans [88], thereby destabilising the conformational development of these proteins during their critical tertiary folding stages [29].

Exposure to different species of metal microcrystal nucleating agent will dictate which specific type of TSE or other neurodegenerative disease will emerge at the end of the day

A complex multifactorial interplay between environmental exposures to the different species of rogue nucleating agent and the different PrP/S-proteoglycan individual phenotypes could dictate which particular strain of TSE will emerge at the end of the day [2]. Likewise, the engagement of various other target proteins — e.g., the beta amyloid protein — in the pathogeneses of a range of other neurodegenerative diseases, suggests that various species of metal pollutant are implicated in the pathogenesis of these disorders; perhaps indicating that each different type of rogue element/metal nucleator will seek out and 'cannibalise' its own complementary target protein, which, in turn, dictates which class of neurodegenerative disorder will emerge at the end of the day. For example, different types of metal intoxication have been proposed as explanations for the origins of Alzheimer's disease, amyotrophic lateral sclerosis, rheumatoid arthritis and Gulf war syndrome, etc. Furthermore, the neuropathology of each of these conditions is differentiated by their own idiosyncratic type of metal-protein crystal deposition; which serves as a diagnostic hallmark in the classification of these diseases [119].

A good example of this is demonstrated in the origins of the aberrant helical structured neurofibrillary tangle that hallmarks the Alzheimer diseased brain [119]. Environmental exposure to the nanocrystals of zinc oxide — which are known to form helical shaped microcrystal structures [120] — could be responsible for the development of the helical shaped metal-protein neurofibrillary tangles that hallmark the Alzheimer's diseased brain.

The metal microcrystal model can account for the mysterious 'heat resistant' and 'transmissible' properties of the TSE agent

When laboratory animals are inoculated with the residual inorganic ash that remains after the heating of TSE diseased brain material up to 600—800 °C, they still develop TSE [11]. This infers that some hitherto unrecognised inorganic, heat resistant component of this ash material must represent the transmissible, pathogenic TSE agent.

This missing link in our understanding of the TSE transmissible agent could be explained by the heat resistant properties of metal microcrystal materials whose crystalline structures will survive heating up to temperatures as high as 500—1000 °C — at which point the thermal agitation of their atoms will be sufficient to shatter the bonds [121]. In this respect, the survival of the atomic structure of the rogue metal microcrystal is synonymous with the survival of any latent pro-oxidant pathogenic capacity (e.g., piezoelectricity, ferromagnetism, radioactivity) that might be stored within the crystal. For example, a magnetic charge will exert a pro-oxidant pathogenic capacity within biological tissues, and this charge could be carried along within any ferrimagnetically ordered crystal contaminant on a permanent basis — unless circumstances change and the crystal is exposed to temperatures that exceed the threshold of its specific curie point; whereupon the magnetic charge would be instantly drained.

It is proposed that the heat resistant, metal microcrystal nucleator represents the relevant component of this ash material that carries the capacity to transmit TSE into the lab animal. Despite evading full recognition to date, these microcrystals are insidiously bonded onto the prion-ferritin proteins in the TSE diseased brain material. So when TSE diseased brain material is artificially inoculated into lab animals, it is the passage of these toxic microcrystals (as opposed to their innocuous transporter proteins) into the recipient animal that represents the relevant causal agent. This provides a plausible explanation for the success of the thousands of TSE transmission experiments that have been enacted over the years [1].

In this respect, exposure to an external environmental source of metal microcrystal can explain the cases of *first generation* TSE; where abnormal prions are generated as a de novo event within the mammalian brain as a direct result of exposure to the relevant environmental causal factors. Furthermore, in whatever low temperature geological or biological, environment the microcrystal has resided, it will permanently retain its pathogenic capacity to seed metal-protein crystals within biological tissues. In this respect, the microcrystal model can explain the induction

of *second generation* TSE in the lab animal via inoculation, as a result of its aforementioned capacity to carry its pathogenicity along with it; thereby inducing second generation TSE in a recipient animal without any need for that animal to be directly exposed to the original environmental source of the pollutant — e.g., the munition factory.

Nitroglycerine crystals; a further candidate that warrants investigation?

In Consideration of the unique properties of glycerine to form crystals under obscure conditions [122,123], Nigel Purdey has suggested that the nitroglycerine component of the munition pollutants observed in the TSE clusters represents another plausible candidate which could serve as the nucleating agent in TSE pathogenesis. In this context, a nitroglycerine-PrP crystal conjugate could act as the progenitor of multireplication, which, in line with Prusiner's theory on prion propagation [1], could induce adjoining PrP glycero-proteins to adopt an identical crystal structure; thereby seeding a progressive domino style conversion, which invokes any contiguous healthy 'non-crystalline' PrP conformations to convert into their rogue crystalline PrPtse isoforms.

This idea is supported by the fact that Glycerine has proved notoriously hard to crystallise, and it is only when the compound is exposed to certain forms of physical agitation (such as the pressure waves of select frequencies of sound, etc.) or ageing, that this can bring about the rare transformation of non-crystallised glycerine into its crystalline form [122,123]. Furthermore, the unusual property of crystallised glycerine is that it can induce neighbouring non-crystallised glycerine molecules to adopt the same crystallised form [123], and, more bizarrely, this induction can even impact glycerine molecules that are separated by the glass walls of storage jars. The strange phenomena of crystallised glycerine carrying out this conversion on non-crystallised molecules at remote locations could possibly shed light upon the pathogenic mechanisms at work in the prion conversion [1] that is purportedly operating in TSE pathogenesis.

This suggestion is supported by the environmental observations in TSE clusters, where the various sources of munitions/propellants that have been identified on the upwind side of the cluster environments, invariably contain a nitroglycerine component. For example, in the autumn of 1999 the Department of Natural Resources initiated a major clean up operation of the nitroglycerine ponds at the 7354 acre Badger Army Munitions Plant in south central Wisconsin [97], which had involved various types of direct incineration and other methods of clean up. A significant clustering of cases of CWD was subsequently identified in the wild deer herd that were grazing a few miles south of the plant around 'Black Earth' in 2001 [94]. This sudden outbreak could not be explained by the conventional theory, since, much like a later outbreak of CWD at the White Sands Missile Range in New Mexico, these fresh cases in Wisconsin represented the first time that CWD had appeared in wild deer outside of Colorado.

Furthermore, in the autumn of 1999, a depth of up to 20 feet of top soil collected from beneath the nitroglycerine ponds at the Badger Plant was sent by a fleet of lorries to a specialist incinerator located at Kimball in Nebraska state [97]. By November 2000, the first cases of CWD had been identified in the deer herd that were resident around Kimball [95].

Conclusion

The results of the analyses of the ecosystems supporting the clusters of sporadic, familial and new variant CJD — as well as sheep scrapie — encompassed in this latest study conducted in Europe, offer additional support in respect of the hypothesis that elevated levels of Ba, Sr, organo phosphorus and/or Ag can provide a pollutant source of rogue metal/element microcrystal which serves as the piezoelectrion nucleating agent in the formation of the aberrant metal-PrP-ferritin fibril crystals that hallmark the neuropathology of the TSE diseased brain. It is also suggested that a radioactive metal species could represent the nucleating agent that seeds the development of the more aggressive new strain TSEs — BSE and vCJD.

Acknowledgements

To Mary Critchley and The Fred Foundation for part funding of this project. Also to Nigel Purdey, Nicola Gazzana, Katsumi Yamada, Ciriaco Ligios, Ralph Berney, Rosemary and Bob Smith for assistance in collection of samples, transport, board/lodging and provision of local knowledge in the different

TSE locations. And to Aster Purdey for secretarial assistance.

References

[1] Collinge J, Palmer MS, editors. Prion diseases. Oxford: Oxford University Press; 1997.
[2] Purdey M. Ecosystems supporting clusters of sporadic TSEs demonstrate excesses of the radical generating divalent cation, manganese, and deficiencies of antioxidant co factors Cu, Se, Fe, Zn. Does a foreign cation substitution at Prp's Cu domain initiate TSE? Med Hypotheses 2000;54(2):278–306.
[3] Purdey M. The manganese loaded/copper depleted bovine brain fails to neutralise incoming shockbursts of low frequency infrasound; the origins of BSE. Cattle Practice 2002;10(4):311–35.
[4] Goudsmit MD, Morrow CH, Asher DM, Yanagihara RT, Masters CL, Gibbs CJ, et al. Evidence for and against the transmissibility of Alzheimer disease. Neurology 1980;30:945–50.
[5] Roitt IM. Essential immunology, third ed. Oxford, UK: Blackwell Scientific Publications; 1977.
[6] Brown P, Will RG, Bradley R, Asher DM, Detwiler L. BSE and vCJD; background, evolution and current concerns. Emerging Infectious Diseases 2001;7(1): 6–16.
[7] BSE Inquiry, vol. 2–10, October 2000; The Stationary Office Limited, PO Box 29, Norwich NR3 1GN.
[8] UK Exports General trade (COD) – Flours and meals of meat and offals/Greaves unfit for human consumption (23011000 exports) (lists importing countries and tonnages received for each year). Dept of Trade, London, UK.
[9] Wilesmith JW, Ryan JBM, Atkinson MJ. BSE; epidemiological studies on the origin. Vet Record 1991;128: 199–203.
[10] Qualitative Analysis of BSE risk factors in the US. USDA: APHIS:VS Animal Health Information, 555 South Howes, Fort Collins, CO 80521, 1991. p. 21.
[11] Brown P, Rau EH, Johnson BC, Bacote AE, Gibbs C, Gajdusek DC. New studies on the heat resistance of hamster adapted scrapie agent: threshold survival after ashing at 600 °C suggests an inorganic template of replication. PNAS 2000;97(7):3418–21.
[12] Cutlip RC, Miller JM, Race RE, Jenny AL, Katz JB, Lehmkuhl BM, et al. Intracerebral transmission of scrapie to cattle. J Infectious Diseases 1994;169:814–20.
[13] Marsh R. Transmissible mink encephalopathy, scrapie and downer cow disease; potential links. Proceedings of third international workshop on BSE. Bethesda, Maryland, December 9 and 10th, 1992.
[14] MAFF; BSE in Great Britain; Progress Reports. June 1988–July 2003 DEFRA, London, UK.
[15] Wrathall AE, Brown KFD, Sayers AR, Wells GAH, Simmons MM, Farrelly SSJ, et al. Studies of embryo transfer from cattle clinically affected by bovine songiform encephalopathy (BSE). Vet Rec 2002;150:365–78.
[16] Dalby J. Soil association survey of the incidence of BSE on organic farms in the UK. 1996. Soil Association, Bristol House, 40-56 Victoria Street, Bristol, BS16BY, UK.
[17] Kirkwood JK, Cunningham AA. Epidemiological observations on spongiform encephalopathies in captive wild animals in the British Isles. Vet Record 1994;135: 296–303.
[18] Sigurdarson S. Epidemiology of scrapie in Iceland and experience with control measures. In: Bradley R, editor. Sub acute spongiform encephalopathies. Brussels and Luxembourg: ECSC EEC EAEC; 1991. p. 233–42.
[19] Palsson PA. Rida in iceland and its epidemiology. Slow transmissible diseases of the nervous system, vol. 1. London: Academic Press; 1979. p. 357–66.
[20] Bradley R, Wilesmith JW. Epidemiology and control of BSE. British Med Bulletin 1993;49(4):932–59.
[21] Lasmezas CI, Deslys JP, Robain O, Jaegly AS, Beringue V, Peyrin JM, et al. Transmission of the BSE agent to mice in the absence of detectable abnormal prion protein. Science 1997;275:402–4.
[22] Brown D, Hafiz F, Glassmith L, Wong BS, Jones I, Clive C, et al. Consequences of manganese replacement of copper for prion protein function and protease resistance. EMBO J 2000;19(6):1180–6.
[23] Wong BS, Chen SG, Colucci M, Xie Z, Pan T, Liu T, et al. Aberrant metal binding by prion protein in human prion disease. J Neurochem 2001;78:1400–8.
[24] Tsenkova RN, Iordanova IK, Toyoda K, Brown DB. Prion protein fate governed by metal binding. Biochem Biophys Res Commun 2004;325(3):1005–12.
[25] Perera WS, Hooper NM. Ablation of the metal ion induced endocytosis of PrP by disease associated mutation of the octapeptide region. Curr Biol 2001;11(7):519–23.
[26] Purdey M. Does an infrasonic acoustic shock wave resonance of the Mn^{3+} loaded/Cu depleted prion protein initiate the pathogenesis of TSE. Med Hypotheses 2003;60(6):797–820.
[27] Purdey M. Elevated silver, barium and strontium in antlers, vegetation and soils sourced from CWD cluster areas: Do Ag/Ba/Sr piezoelectric crystals represent the transmissible pathogenic agent in TSEs? Med Hypotheses 2004;63:211–25.
[28] Brown DR. Prion and prejudice; normal protein and the synapse. Trends Neurosci 2001;24(2):85–90.
[29] Knaus KJ, Morillas M, Swietnicki W, Malone M, Surewicz WK, Yee VC. Crystal structure of the human prion protein reveals a mechanism for oligomerization. Nat Struct Biol 2001;8(9):770–4.
[30] Choudhury H, Cary R. Concise international chemical assessment document: 33 – barium and barium compounds. Geneva: WHO; 2001.
[31] Petering HG. Pharmacology and toxicology of heavy metals: silver. Pharmac Ther A 1976;1:127–30.
[32] Perriman N, Bernfield M. Nature 2000;404:725–8.
[33] Gianutosos G, Morrow GR, Morris JB. Accumulation of Mn in rat brain following intranasal administration. Fund Appl Toxicol 1997;37(20):102–5.
[34] Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, et al. Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect 2001;109(4):547–51.
[35] Ascher M, Ascher JL. Manganese Neurotoxicity; cellular effects and blood brain barrier transport. Neurosci Biobehav Rev 1991;15:333–40.
[36] Mayland HF, Florence AR, Rosenau RC, Lazar VA, Turner HA. Soil ingestion by cattle on semi-arid range as reflected by titanium analysis of feces. J Range Manage 1975;28(6):448–52.
[37] Arthur WJ, Alldredge AW. Soil ingestion by mule deer in North Central Colorado. J Range Manage 1979;32(1): 67–71.
[38] Bastian F. CJD and other transmissible encephalopathies. Mosby Year book 1991 St Louis.

[39] Becker RO, Selden G. The Body Electric: electromagnetism and the foundation of life. New York: Quill William Morrow; 1985.
[40] Halliwell B, Gutteridge JMC, editors. Free radicals in biology and medicine. 2nd ed.. Oxford, UK: Clarendon Press; 1989.
[41] Piening N, Weber P, Giese A, Kretzschmar H. Breakage of PrP aggregates is essential for efficient autocatalytic propagation of misfolded prion protein. BBRC 2005;326:339–43.
[42] Takeuchi Y, Matsumoto T, Ogata E, Shishiba Y. Isolation and characterization of proteoglycans synthesized by mouse osteoblastic cells in culture during the mineralization process. Biochem J 1990;266: 15–24.
[43] Mani K, Cheng F, Havsmark B, Jonsson M, Belting M, Fransson L. Prion or amyloid-b-derived Cu (11)- or free Zn (11)-ions support S-nitroso-dependent autocleavage of glypican-1 heparan sulphate. J Biol Chem 2003(5).
[44] Perez M, Wandosell F, Colaco C, Avila J. Sulphated glycoaminoglycans prevent neurotoxicity of a human prion protein fragment. Biochem J 1998;335:369–74.
[45] Shyng S-L, Lehmann S, Moulder KL, Harris DA. Sulfated glycans stimulate endocytosis of the cellular isoform of the prion protein in cultured cells. The Am Soc Biochem Mol Biol 1995;270(50):30221–9.
[46] Jarrett JT, Lansbury PT. Seeding one dimensional crystallization of amyloid; a pathogenic mechanism in Alzheimer's disease and Scrapie. Cell 1993;73:1055–8.
[47] Gajdusek DC. Spontaneous generation of infectious nucleating amyloids in the transmissible and nontransmissible cerebral amyloidoses. Mol Neurobiol 1994;8(1):1–13.
[48] Agrimi U, Riu G, Cardone F, Pochiari M, Caramelli M. Epidemic of TSE in sheep and goats in Italy. Lancet 1999;353:560–1.
[49] Underwood EJ. Trace elements in human and animal nutrition. 4th ed.. New York: Academic Press; 1977.
[50] Miyakawa T, Inoue K, Iseki E, Kawanishi C, Sugiyama N, Onishi H, et al. Japanese CJD Patients exhibiting high incidence of the E200k PRNP mutation and located in the basin of a river. Neurol Res 1998;20:684–8.
[51] Akai J, Ishihara O, Higuchi S. CJD in Japan: an epidemiological study done in a select prefecture between 1976 and 1986. Neuroepidemiology 1989;8:32–7.
[52] D'Alessandro M, Petraroli R, Ladogana A. Pocchiari M. High incidence of CJD in rural Calabria. Lancet 1998;352:1989–90.
[53] Caramelli M, Ru G, 2002. CEA Centre di referenza encephalopatie animali, Istitutoooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy.
[54] CJD surveillance in the UK; 1998–2003. Annual Reports of the National CJD Surveillance Unit, Western General Hospital, Edinburgh, EH42XU.
[55] Cartwright, TC. Birds eye wartime; Leicestershire 1939–1945 TCC publications, Hinckley, Leicestershire. 2002 ISBN 0953449718.
[56] Rosie G. Sunday herald, 9th February 2003. www.sunday-herald.com/np/31247.
[57] Stockholm International Peace Research Institute. The problem of chemical and biological warfare. A study of the historical, technical, military, legal and political aspects of CBW and possible disarmament measures. vol 1. The rise of CB weapons. New York: Humanities Press; 1971. p. 119 ff 305.
[58] Ospar Commission. Overview of past dumping at sea of chemical weapons and munitions in the Ospar maritime area; Biodiversity series. 2004 ISBN 1-904426-54-9. Available from: www.ospar.org/documents/dbase/publications/p00144-2004 dumping at sea of chemical weapons pdf.
[59] Kirby A, ''UK's undersea ticking timebomb''. BBC News website. Available from: www.news.bbc.co.uk/1/hi/sci/tech/4032629.stm.
[60] Fisheries Research services report no 15/96. Surveys of the Beaufort dyke explosives disposal site; November 1993-July 1996. Final report. November 1996. The Scottish Office, Agriculture, Environment and Fisheries dept, Marine Laboratory, Victoria Road, Aberdeen, Scotland. Available from: www.marlab.ac.uk/FRS.web/uploads/documents/frsr1596.pdf.
[61] Usage of Raw materials in Animal Feeds in Great Britain 1984–1995; DEFRA statistics, Foss House, Kings Pool, 1–2 Peasholme Green, York Y01 2PX, UK.
[62] The ENDS reports; 357, Oct 2004; 353, June 2004; 342 Jul, 2003; 299, Dec 1999; Environmental Data services Ltd, Finsbury Business Centre, 40 Bowling Green Lane, London, ECIR ONE, UK. Available from: www.endsreport.com.
[63] The ENDS reports; 253, Feb 1996; 233, June 1994; Environmental Data services Ltd, Finsbury Business Centre, 40 Bowling Green Lane, London, ECIR ONE, UK. Available from: www.endsreport.com.
[64] The Portland Cement Association. Use of cement Kiln dust for agricultural applications; a bibliography of publications available in the PCA library. Available from: www.cement.org/pdf_files/LB04.pdf.
[65] Medway Libraries; Headcorn airfield; activity and events 1944 onwards. BBC; WW2 People's war. Article ID; 3098540, 7th October 2004. www.bbc.co.uk/dna/ww2/A3098540.
[66] Study into noise mitigation measures at Lashenden (Headcorn) aerodrome. Dept of Transport. 1996. Available from: www.dft.gov.uk/stellent/groups/dftn_503308-04.hcsp.
[67] Woodnough JL. Attlebridge Diaries: the history of the 466th Bombardment group. Sunflower University Press; 1985, ISBN 0897450671.
[68] Colombo F. MAFIA dominates garbage industry. Tierramerica, 2004. Available from: www.tierramerica.net/2003/0623/iarticulo.shtml.
[69] Edmondson G, Carlisle K. Italy and the Eco-mafia. Business Week Magazine. January 27th, 2003. Available from: www.businessweek.com/magazine/content/03_04/63817015html.
[70] Mangano A, La Mafia a Sigonella. Lecture; 25th November 2001. Terrelibere.org. Available from: www.terrelibere.it/counter.php?riga=41&file=mafiasigonella.htm.
[71] Prinz M, Harlow G, Peters J, editors. Rocks and minerals. New York: Simon and Schuster; 1977.
[72] The United States Army Air Force in World War two; Combat chronology of the US Army Air forces in WW2. July 1943. Available from: www.usaaf.net/chron/43/jul43.htm.
[73] Eldredge WJ. 2rd Chemical Mortar battalion in Sicily 1943.World War Two Magazine. February 1999.
[74] Alpers MP. Kuru. In: Attenborough RD, Alpers MP, editors. Human biology in Papua New Quinea. New York: Clarendon Press; 1992 [Chapter 16].
[75] Hall M, Kimura H. Quantitative EDXRF studies of Obsidian sources in Northern Hokkaido. J Archaeological Sci 2002;29:1–8.
[76] Mitrova E. Some new aspects of CJD epidemiology in Slovakia. Euro J Epidemiol 1991;7(5):439–49.

[77] Mitrova F, Bronis M. Clusters of CJD in Slovakia; the first statistically significant temporo-spatial accumulations of rural cases. Eur J Epidemiol 1991;7(5):450–6.
[78] Mineral tolerance of domestic animals (1980). National Academic Press. Barium, p. 54-9; Strontium p. 459–65.
[79] Eisenbud M, Gesell T. Environmental radioactivity. 4th ed.. London: Academic Press; 1997.
[80] Shacklette A. In: Oehme FW, editor. Toxicity of heavy metals in the environment Part 1. New York: Marcel Dekker, Inc.; 1978. p. 56–9 [Chapter 3].
[81] Mazzeo A, Missili e strategie nell'isola portaerei della NATO. Lecture 17th April 2004. Terrelibere.org. Available from: www.terrelibere.it/counter.php?riga=168&letture=750&file=.
[82] Mazzeo A, Mediterraneo mare di guerra. Lecture 29th Febuary 2004 Terrelibere.org. Available from: www.terrelibere.it/counter.php?riga=164&letture=1144&file=.
[83] Daly MJ, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, Venkateswaran A, Hess M, Omelchenko MV, Kostandarithes HM, Makarova KS, Wackett LP, Fredrickson JK, Ghosal D. Accumulation of Mn(II) in *Deinococcus radiodurans* Facilitates Gamma-Radiation Resistance Science 5 November 2004;306:1025–28.
[84] Raymond KN, Telford JR. Bioinorganic Chemistry: an inorganic perspective of life. NATO ASI series. Dordrecht: Kluwer Academic publishers; 1995. p. 25–37.
[85] Cho HJ, Grieg AS, Corp CR, Kimberlin RH, Chandler RL. Nature (London) 1977;267:459–60.
[86] Jackson GS, Murray I, Hosszu LP, Gibbs N, Waltho JP, Clarke AR, et al. Location and properties of metal-binding sites on the human prion protein. PNAS 2001;98(15):8531–5.
[87] Grant D, Long WF, Williamson FB. A potentiometric titration study of the interaction of heparin with metal cations. Biochem J 1992;285:477–80.
[88] Nader HB, Takahashi HK, Guimaraes JA, Dietrich CP. Heterogeneity of heparin: characterisation of one hundred components with different anti-coagulant activities by a combination of electrophoretic and affinity chromatography methods. Int J Biol Macromolecules 1981;3(6):356–60.
[89] Pais I, Benton Jones J. The handbook of trace elements. Florida: St Lucie Press; 1997.
[90] Stanford CM, Jacobson PA, Eanes ED, Lembke LA, Midura RJ. Rapidly forming apatitic mineral in an osteoblastic cell line (UMR 106_01 BSP). Am Soc Biochem Mol Biol 1995;270(16):9420–8.
[91] Naughton DP. Iron (111)-mediated intra-articular crystal deposition in arthritis: a therapeutic role for iron chelators. Med Hypotheses 2001;57(1):120–2.
[92] Crom R, Cluster of CJD cases in Tucson. Report; The epidemic Intelligence Service of the Arizona Department of Health Services, Pheonix, 1990.
[93] Williams ES, Young S. Spongiform encephalopathy in cervidae. Rev Sci Tech Off Int Epiz 1992;11(2):551–67.
[94] Department of Natural resources; State of Wisconsin Division. Chronic Wasting Disease eradication, 2004. Available from: www.dnr.state.wi.us/org/land/wildlife/Whealth/issues/Cwd/dez_2003_pos.jpg.
[95] Bischot R, Nebraska Game and Park commission. CWD test results. Western Nebraska, November 2000–April 2000. Available from: www.outdoornebraska.org/wildlife/cwd/cwdinfo.html.
[96] Purdey M. Elevated levels of Ferrimagnetic metals in Foodchains supporting the Guam cluster of neurodegeneration. Do metal nucleated crystal contaminants evoke magnetic fields that initiate the progressive pathogenesis of neurodegeneration? Med Hypotheses 2004;63(5):793–809.
[97] Badger Army Ammunition Plant; Environews Reports ; 1998 to present day. Available from: www.badger-aap.org/about.htm.
[98] Hourrigan JL, Klingsporn AL. Scrapie: studies on vertical and horizontal transmission. In: Gibbs CJ, editor. Bovine spongiform encephalopathy: The BSE Dilemma. Serono Symposia USA, Norwell, Massachusetts. New York: Springer-Verlag; 1996 [Chapter 5].
[99] Sigmund L. Race against the dying. London: Pluto Press; 1980.
[100] Another Leics Bomber Crash – seven killed at Queniborough. Leicestershire Mercury: p1, Thursday Febuary 21st 1946.
[101] Abdel Aziz MI, Radford J, McCabe J. Finningley Airport Health Impact Assessment. Annex 4: the evidence – base for potential impacts. Doncaster Health Authority September 2000. Available from: www.phel.gov.uk/hiadocs/79_finningley_airport_hiareport.pdf.
[102] Will RG, Matthews WB, Smith PG. A retrospective study of CJD in England and Wales 1970-1979: 11 Epidemiology. J Neurol Neurosurg Psychiatry 1986;49:749–55.
[103] Trabattoni G, Lechi A, Bettoni G, Macchi G, Brown P. Considerations on a group of13 patients with CJD in the region of Parma, Italy. Eur J Epidemiol 1990;6(3):239–43.
[104] Devlin TM. Textbook of Biochemistry with Clinical Correlations. 3rd ed.. New York: Wiley-Liss; 1992.
[105] Paterson CR. Essentials of human biochemistry. London: Pitman Publishing; 1983.
[106] Frausto Da Silva JJR, Williams RJP. The biological chemistry of the elements. 2nd ed.. Oxford: Oxford University Press; 2001.
[107] Gajdusek DC. Hypothesis: interference with axonal transport of neurofilament as a common pathogenetic mechanism in certain diseases of the CNS. New England J Med 1985;312(11):714–8.
[108] Kan M, Wang F, Kan M, To B, Gabriel JL, McKeehan WL. Divalent Cations and heparin/Heparan Sulphate cooperate to control assembly and activity of the fibroblast growth factor receptor complex. The Am Soc Biochem Mol Biol 1996;271(42):26143–8.
[109] Leteux C, Chai W, Nagai K, Herbert C, Lawson A, Feizi T. 10E4 antigen of scrapie lesions contains an unusual nonsulphated heparin motif. J Biol Chem 2001;276(16):12539–45.
[110] Schrooten I, Cabrera W, Goodman WG, Dauwe S, Lamberts LV, Marynissen R, et al. Strontium causes osteomalacia in chronic renal failure rats. Kidney Int 1998;54(2):448.
[111] Verberckmoes SC, De Broe ME, D'Haese PC. Dose-dependent effects of strontium on osteoblast function and mineralization. Kidney Int 2003;64(2):534.
[112] Annual Animal Health Reports; Warble Fly. 1980–1995. MAFF, Toby Jug Site, Tolworth, Surrey, UK.
[113] Purdey M. Are Organo phosphate pesticides involved in the causation of BSE. J Nutritional Med 1994;4(1):43–82.
[114] Purdey M. High dose exposure to systemic phosmet insecticide modifies the phosphatidylinositol anchor on the prion protein; the origins of new variant transmissible spongiform encephalopathy. Med Hypotheses 1998;50(2):91–111.
[115] Tarry DW. Progress in warble fly eradication. Parasitol Today 1986;2(4):111–6.
[116] Gordon I, Abdulla EM, Campbell IC, Whatley S. Phosmet induces upregulation of surface levels of cellular prion protein. Neuroreport 1998;9(7):1391–5.

[117] Review of the Origin of BSE; Gabriel Horne Committee, DEFRA London, UK 5th July 2001.
[118] Organophosphorus Insecticides; Environmental Health Criteria 63. IPCS International Programme on chemical safety. World Health Organisation, Geneva; 1986.
[119] Calne DB, Eisen A, McGeer E, Spencer P. Alzheimer's disease, Parkinson's disease, and motor neurone disease: abiotrophic interaction between ageing and environment. A Hypothesis. Lancet 8th November 1986. p. 1067-70.
[120] Wang Science March 2001 Zinc Nanosprings.
[121] Kittel C. Introduction to solid state physics. seventh ed.. New York: John Wiley and Sons; 1995.
[122] Oparin AI, The origin of life on earth Oliver and Boyd, Edinburgh 1957.
[123] Gibson GE, Giauque WF. 3rd law of thermodynamics. J Am Chem Soc 1923;45:93—7.