



**PAPER No: SEAC 76/5**

**Department of Health's Annual Summary of TSE Related Research  
(April 2001-March 2002)**

**Issue**

Further to a request from SEAC, Members will be presented with an update on the DH TSE Research Programme (2001 to 2002).

**Background**

The DH funds approximately £5m on research. This represents approximately 20% of the total annual government spend on TSE research.

**Advice Sought from the Committee**

This paper is provided for information only.

## DEPARTMENT OF HEALTH

### ANNUAL SUMMARY OF TSE-RELATED RESEARCH

APRIL 2001 – MARCH 2002

#### SUMMARY OF ACTIVITIES

The Department, through its Research and Development Directorate, commissions research to inform its policy needs concerning human TSEs in the following areas

- Epidemiology and surveillance
- Blood safety
- Tissue infectivity and strain typing
- Diagnosis and detection
- The development and assessment of therapeutic drugs
- Decontamination.

During the period 1/4/01-31/3/02 one contract was completed, 56 were still in progress and 18 new contracts were commissioned.

The expenditure for this period was divided among the 6 main research topics as follows

Epidemiology and surveillance	£1,007,000
Blood safety	£73,000
Tissue infectivity and strain typing	£481,000
Diagnosis and detection	£1,271,000
Development and assessment of therapeutic drugs	£1,473,000
Decontamination	£896,000
<b>Total expenditure for the year</b>	<b>£5,201,000</b>

A complete list of projects is attached (Appendix A) and further details can be found on the MRC website, ([www.mrc.ac.uk/tse\\_2c.htm](http://www.mrc.ac.uk/tse_2c.htm)) or in the DH National Research Register (NRR) ([www.doh.gov.uk/research/nrr.htm](http://www.doh.gov.uk/research/nrr.htm)) and DH ReFeR ([www.doh.gov.uk/research/rd3/information/findings.htm](http://www.doh.gov.uk/research/rd3/information/findings.htm)) databases.

## **EPIDEMIOLOGY AND SURVEILLANCE**

The number of cases of vCJD reported each year continued to rise, but mathematical analysis of the trend indicated that the rate of increase was beginning to slow. Nation-wide surveys of atypical dementia in the elderly and progressive intellectual and neurological deterioration (PIND) in young children failed to provide any evidence that cases were being missed in either the elderly or the very young. Geographically associated cases of vCJD in Leicestershire and in the Southampton area were investigated and evidence was accumulated to suggest that historical (now illegal) local butchery practices might have given rise to the cases in Leicestershire. Some of the Southampton cases had been given polio vaccine from the same batch, but as the batches were very large it could not be concluded that these cases were linked to vaccination.

A fundamental re-assessment of the case control study was made in an attempt to improve the recruitment of suitable controls. After extensive peer review and consideration by the SEAC Epidemiology Sub-Group, a revised protocol was agreed and implemented.

Although the next phase of the retrospective surveys of stored appendices and tonsils and the pilot study of freshly collected tonsils were not due to be completed until later in 2002, wide-ranging consultations about a nation-wide survey were commenced. The logistics subgroup of the DH/MRC steering committee concluded that fresh tonsil tissue would be the most appropriate samples to analyse and that the collection should be carried out as soon as possible to maximise the analysis of tissue from those most likely to be exposed to the BSE agent. The difficult ethical issues associated with this nation-wide survey were debated extensively, although no clear consensus was reached. A report containing the recommendations of the two groups was submitted to the CMO (England) in March 2002.

## **BLOOD SAFETY**

Analysis of whole blood and blood fractions from patients with vCJD have failed to detect either infectivity (by intracerebral challenge in mice) or PrP<sup>Sc</sup> (by Western blotting). However the earlier reports of BSE transmission in sheep by blood transfusions from orally infected animals were confirmed. In addition reports of scrapie transmissions by blood transfusions from infected hamsters was also reported by American workers. No results were available from experiments in primates.

Experiments to monitor the retention of infectious prions during blood fractionation had been delayed due to lack of facilities for research in experimental animals. This has now been resolved and results are expected in the summer of 2003. The studies to monitor adverse or beneficial effects of leukodepletion are expected to report in the autumn of 2002.

## **TISSUE INFECTIVITY AND STRAIN TYPING**

Routine histological and immunohistochemical analysis of post mortem tissue from vCJD patients has not yet revealed evidence for significant strain variation. Similarly, passage in mice of a smaller number of tissue samples has also shown no evidence for variation in the agent causing vCJD. Estimates of infectivity in the brains of vCJD patients indicate that they are similar to those found in BSE-infected calves, but levels in tonsils and spleen are about 1,000 times lower. Newly commissioned research will analyse levels of infectivity in spinal cord, CSF, appendix, lymph nodes, peripheral nerve, dorsal root ganglia, trigeminal ganglia and bone marrow and it is intended to rapidly commission further work to study infectivity in eye, dental pulp, gingiva, distal ileum, skeletal muscle, kidney, adrenal gland, heart, liver and lung.

Similar work analysing levels of PrP<sup>Sc</sup> by Western blot have failed to detect PrP<sup>Sc</sup> in peripheral nerves, appendix<sup>1</sup>, white blood cells, heart, lung, pancreas, kidney, liver, skin and the anterior part of the eye. However PrP<sup>Sc</sup> has been detected in brain, spinal cord, posterior parts of the eye, thymus, lymph nodes, tonsil, spleen and occasionally in the adrenal glands and in rectal tissue.

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<sup>1</sup> However, recent histopathological studies of appendices have shown positive results for the presence of abnormal prion protein (Hilton, *et.al.*, 2002, *BMJ* **325**, 633-634)

## DIAGNOSIS AND DETECTION

The diagnosis of pre-clinical infection remains a major target of the DH programme of CJD-related research. Although no suitable diagnostic assay has yet been announced, research in this area received a major boost through the co-ordinated call for research proposals on diagnosis, co-ordinated by the MRC. Through this call DH has undertaken to support the following research programmes.

- 1) Development of rapid visual and epimicroscopy techniques – Professor C W Keevil, University of Southampton
- 2) Heart rate variability as an aid to diagnosis of vCJD – Dr C Pomfrett, University of Manchester
- 3) Novel application – specific monoclonal antibodies to PrP – Dr M Head, NCJDSU, Edinburgh
- 4) Tau protein as a diagnostic tool for vCJD – Dr A Green, NCJDSU, Edinburgh
- 5) Screening blood for high risk donations – Dr M Clinton, Roslin Institute
- 6) Removal of infective protein residues from medical instruments – Dr R L Baxter, University of Edinburgh
- 7) To create a validatable procedure to check protocols for the decontamination of surgical instruments – Dr H Reid, Moredun Institute.

In addition to the programmes supported by the UK government there is extensive activity in this area outside the UK. During the period of this report two potentially important developments were announced. The first was the report by Dr Soto and his colleagues of a method for amplifying small amounts of abnormal prion protein in test samples. However attempts to repeat this important finding have either been unsuccessful or have reported low levels of amplification. The second report came from a group working in Israel who claimed to be able to detect disease-specific prions in the urine of experimentally infected animals. The Department has recently

commissioned research at the NCJDSU to determine whether similar results can be obtained using urine from CJD patients.

Although it is almost universally recognised that it is very important to develop a non-invasive test, which can detect individuals who are infected with CJD but not displaying clinical symptoms, opinion on how to use such a test is divided. For example, would it be ethical to withhold information from individuals who give material for research, or as part of a blood or tissue donation? Conversely would it be helpful to inform people that their blood or tissue sample was positive when the meaning of a positive result is unknown, there is no cure or treatment and no recommendation can be given on altering lifestyles? Consequently it has been decided to debate these and several other related ethical issues at a seminar to be arranged in the autumn of 2002.

## **THE DEVELOPMENT AND ASSESSMENT OF THERAPEUTIC DRUGS**

The low level of activity in the field of CJD drug development has concerned DH for several years. During the past 12 months however the following new research contracts have been commissioned.

1. Discovery of novel therapeutic drugs for prion delivery – Dr B Chen, Cranfield University.
2. Development of lead peptides as anti-TSE agents – Dr I H Gilbert, Welsh School of Pharmacy.
3. Novel therapeutic drug agents for prion disease – Dr N Raven, CAMR.

In addition to the expanded programme of research funded directly by DH, the MRC Prion Unit presented encouraging new results at the UK TSE Funders workshop in Durham. Initial work screening a chemical library from Glaxo-Smith-Kline (GSK) had identified several potential lead compounds and research describing the therapeutic use of anti-prion antibodies in experimental animals was reported. The MRC Prion Unit also undertook a substantial amount of work to develop a clinical

trial protocol for quinacrine, in conjunction with the MRC Clinical Trials Unit. This protocol has been subject to peer review through the MRC and a decision is expected early in September 2002. The scientific reviewers recommended that the opinions of patient groups should be sought before a final decision is made and a one-day workshop with them was held in July 2002. In addition it was recommended that current clinical experience of quinacrine use in CJD therapy should be reviewed before a trial is started and this data is being collected. During the discussions over the design of clinical trials the difficulties of working with very small groups of patients were raised. Consequently a CJD Therapy Advisory Group has been set up, chaired by Sir Michael Rawlins, to consider general strategic and ethical issues associated with the assessment of a number of potential therapeutic treatments in small numbers of patients with terminal disease.

## **DECONTAMINATION**

Effective decontamination of surgical instruments remains a cornerstone in the Department's strategy to prevent the spread of human TSEs. Recently £200m has been allocated to improve the quality of hospital sterile service departments and to date DH has committed over £5m to research on the detection and inactivation of prions bound to solid surfaces. This work is overseen by the Working Group for Research into the Decontamination of Surgical Instruments, chaired by Professor Don Jeffries and recent progress is summarised below.

### **Assessing damage to instruments from alkali autoclaving**

Tokens (discs of metal) prepared from various grades of stainless steel have been subject to typical hospital sterilisation protocols or to alkaline autoclaving. These protocols have been designed to simulate the conditions experienced by the average surgical instrument over 12 months. Surface damage has been assessed by visual inspection, scanning white light interferometry and electron microscopy. In general, apart from some surface discoloration, most stainless steels suffer little surface damage when subject to these conditions and when analysed by these methods. The only exception being some grades of steel with a low nickel content, used in the manufacture of some of the cheaper imported instruments.

Recently commissioned research will concentrate on the evaluation of a commercial “alkali autoclave” and in determining whether less severe procedures involving alkali and heat treatment will be efficacious.

### **Tissue loading on instruments from a typical hospital SSD (Sterilizing services Department), before and after processing**

Examination of instruments coming to a typical hospital SSD revealed that most instruments were contaminated with 60-100 mgs of tissue, rather less (~30mgs) for brain tissue. These levels were consistent with the values assumed for the risk assessment calculations performed by EOR (Economics and Operational Research). Using several detection methods commercial enzymic cleaners used in hospital SSDs were shown to remove greater than 99% of protein bound to surgical instruments after a single wash cycle. However further cycles removed little more than 1% of the remaining protein. However, once proteins had been dried onto instruments they were more difficult to remove and some proteins such as fibrinogen corroded steel surfaces during prolonged contact. It was of interest to note that most of the commercial cleaners used in hospital SSDs operate at pH11 or above. These studies will shortly be extended to include dental instruments.

### **Detection of prions on surfaces**

The ability of several new commercial dyes to detect proteins bound to stainless steel surfaces has been studied and, OPA/NAC (commercial names for dyes), gave a linear response from 0 – 2.5µg/ml; with reproducible results to within 1% for most proteins at a sensitivity of around 100ng/ml. Future studies will investigate MALDI-ToF (mass spectrometry) to analyse proteins on steel surfaces.

Workers at the Institute of Biotechnology in Cambridge have developed Magnetic Acoustic Resonance Sensors (MARS) for the detection of prions on surfaces. Initial studies were able to demonstrate the binding of prions to Ferritic steel, measured by EDX (Energy Dispersive X-ray analysis). However it was not possible to improve the sensitivity of MARS as high acoustic frequencies were absorbed by the steel. Other studies using Auger electron spectroscopy had demonstrated that the surface of stainless steel was essentially an oxidised layer of chromium. Consequently it was

possible to simulate this material by coating optically polished silica glass with chromium and this material was not subject to the limitations of stainless steel itself. Using this technology prion concentrations as low as 62.5µg/ml have been detected, although the detection limit of this technology has not yet been determined. The efficacy of UV-ozone in cleaning these materials has been assessed and initial results indicate that this method can remove 95% of the total protein bound to the contaminated surface. An ELISA assay using 5mm steel discs has been developed to confirm the results obtained from MARS and this assay can detect PrP at between 100 and 10ng/ml.

The CAMR group has demonstrated the proof of principle for the development of a high sensitivity ELISA, based on thermostable adenylate kinases and capable of detecting material bound to solid surfaces. This assay is capable of detecting material at femtomolar or attomolar concentration, but a suitable prion-specific antibody for use in this ELISA has not yet been identified. In addition a number of better thermostable enzymes from several thermophilic bacteria and archaea have been identified.

### **Novel chemical and enzymic inactivants**

CAMR is evaluating a series of highly efficient thermostable proteins, in conjunction with the biotechnology company GENENCOR.

Workers at NPU are studying the biochemical properties of abnormal prion protein which correlate with the relative differences in resistance to inactivation observed in various strains of TSEs. This group has also confirmed earlier observations that the disappearance of PrP<sup>res</sup> does not always correlate with inactivation and thus this may not always be a suitable surrogate marker for infectivity. Future work by this group will extend these studies to evaluate the ability of a number of enzymes, detergents and chaotropic agents to enhance the inactivation of TSEs. The ability of current hospital autoclave protocols to inactivate vCJD will also be studied.

## **Detecting infectious prions bound to solid surfaces**

Members of the MRC Prion Unit have demonstrated that stainless steel wires exposed to the brains of scrapie-infected mice or to brain homogenates for as little as 5 minutes can efficiently transmit infectivity to indicator mice. The recipient mice can be exposed to the infectious wires for as little as 30 minutes for disease to be induced. These workers have also shown that infectivity bound to the wires persists for far longer in the brain than injected homogenates and prions remaining bound to the wires can transmit disease efficiently. Similar results are obtained with wires exposed to animals in both the preclinical and clinical stages of disease and with wires treated with infected spleens.

Some chemicals, such as sodium hydroxide and sodium isothiocyanate, were shown to efficiently remove infectivity bound to the wires, but formaldehyde did not.

## **Recently commissioned research**

Validation of the WR<sup>2</sup> alkali autoclave (WR<sup>2</sup> is the commercial name for the company) - Robert Somerville and Karen Fernie; BBSRC Neuropathogenesis Unit. (Contract Number 7042).

Inactivation by gas plasmas – David Perry: CSMA. (Contract Number 7389).

## **Novel approaches under discussion**

In addition to the research funded directly by DH discussions have taken place with commercial companies concerning the following technologies:

High energy radiation, as used to sterilise disposable laboratory and clinical equipment – Varian UK

Ozone sterilisers – produced by the Canadian company TSO<sub>3</sub>

Modified protocols based on peracetic acid – Steris Inc.

## **Science and Engineering Group**

From the work outlined above it appears that a number of DH contractors are nearing the stage where they will be able to offer novel reagents or protocols for formal evaluation. In addition it is apparent that at least 3 commercial companies, Varian, TSO<sub>3</sub> and Steris have reagents or technologies which are also close to formal evaluation. Consequently it was agreed at the January 2002 meeting of the Working Group on the Decontamination of Surgical Instruments to set up a Science and Engineering Group. This group, chaired by Darryn Kerr of NHS Estates, will consider ways in which these new processes can be formally evaluated and, where appropriate, brought into practice by the NHS. A pilot group to decide the remit and composition of this group will meet in May 2002.

**CURRENT STATUS OF TSE RESEARCH**

**DEPARTMENT OF HEALTH**  
**RESEARCH AND DEVELOPMENT DIRECTORATE**

**Epidemiology**

<b>Lead Researcher</b>	<b>Contract number</b>	<b>Research subject</b>	<b>Status</b>
R.G. Will	6407	Core support for CJDSU	continuing
R.G. Will	6981	Risk factors for CJD	ends in 2002
C. Verity	6443 & others	Co-ordinated national study of PIND	extn. to 2003.
M. Coleman	6462	Occupational risk of CJD	completed
P.G. Smith	6463	Spatial clustering of CJD	completed.
A. Swerdlow	6464	CJD associated with HGH treatment	completed, but final data analysis still in progress
J.W. Ironside	6469 & others	Co-ordinated national study of Atypical dementias	ends in 2002.
M. Esiri	6470	Survey of autopsy brain samples	ends in 2002.
M.P. Coleman	6471	Survey of national mortality records in England.	completed.
J. Lowe	6472	Model for neuropathological Surveillance of the elderly.	Ends in 2002
R. Salmon	6473	Survey of national mortality records in Wales.	completed.
J. Collinge	6958	Prospective study of tonsil tissue	ends in 2002*
D. Hilton	6963	Retrospective study of tonsil and appendix tissue from S.W. England.	ends in 2003 *
J.W. Ironside	6982	Retrospective study of tonsil and appendix tissue from Scotland	ends in 2002 *

C. Lee	6984	Prevalence studies in haemophiliacs	ends in 2004
P.G. Smith	7127	Mathematical models for predicting future cases of vCJD.	ends 31/3/03
A. Ghani	7390	Predicting the vCJD epidemic	funding agreed
H. Ward	7400	Continuation of the case control study	funding agreed

\* These studies are co-ordinated and monitored by a MRC/DH Steering Group, chaired by Professor Boryseiwicz.

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### **Strain typing**

<b>Lead Researcher</b>	<b>Contract number</b>	<b>Research subject</b>	<b>Status</b>
J. Collinge & C. Bostock	6406	Additional animal housing at Northwick Park and IAH	completed
M. Bruce	6426	Strain typing of CJD by transmission to mice.	completed. Report awaited
J. Collinge	6491	Core funding for pathogenesis studies using transgenic mice.	ends 31/12/03
P. Minor	7142	Review of the use of transgenic animals In CJD research.	completed
J. Collinge	7147	Additional animal facilities for research on CJD pathogenesis.	ends in 2004
J. Collinge	7497	Transgenic animal facility at DSTL	Building completed
M. Bruce	7303	Assessment of infectivity in tissues from v CJD patients.	ends 2003
R. Will	7408	PRNP regulatory sequences	Contract issued

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## Diagnostics

Lead Researcher	Contract number	Research subject	Status
E. Miller	6466	Collection of CSF to evaluate CJD diagnostic protocols.	completed
R. Eglin	6888	Development of a diagnostic test using modified Congo red dye.	report due
J.Collinge	6959	Development of monoclonal antibodies against prions.	ends 2002
P. Minor	7071	Establishment of a national resource for CJD biological materials.	current support ends 31/10/03
M. Head	7175	Detection of prions by Capillary electrophoresis	ends 2004
J. Collinge	7498	Further funding for CJD diagnostics.	Funding agreed for 2001/02
T. Barrowcliffe	7341	Detecting prions in blood by ICE	ends 2003
D. Collie	7369	Validation of vCJD diagnosis by MRI	ends 2004
M. Head	007/0064	Detection of prion protein in the urine of CJD patients	funding agreed
C. Pomfett	007/0074	Heart rate variability as an aid to the diagnosis of vCJD	funding agreed
A. Green	007/0075	tau protein as a diagnostic tool for vCJD	funding agreed
I. MacGregor	007/0076	Novel application-specific monoclonal Antibodies to PrP	funding agreed
M.Clinton	007/0078	Screening blood for high risk donations	funding agreed

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## Therapeutics

H. Sharma	7128	Pharmacokinetics of pentosan Polysulphate in mice and man	ends 2003
A. Williams		Cox 2 inhibitors as therapeutic Agents against CJD	Under review
N. Raven	7381	Development of novel therapeutics	Contract sent out
B. Chen	7534	Discovery of novel drugs for TSEs	Contract sent out
I. Gilbert	0081	Development of lead peptides as anti-TSE agents.	Started April 2002

## Safety of blood and blood products

Lead Researcher	Contract number	Research subject	Status
C. Bostock	6713	Studies on the transmission of TSEs by whole blood or leukocytes. (sheep)	ends in 2005
M. Bruce	6886	Studies on the transmission of TSEs by whole blood or leukocytes. (human)	completed report awaited
T. Wallington	6925	Studies of the effect of leukocyte depletion on prions.	ends in 2001
L. Williamson	6936	Effect of leukocyte depletion on post-operative care.	co-ordinated with 6925.
C. Farquhar	6978	Use of pentosan polysulphate as an anti-CJD agent in blood transfusion.	ends in 2003
D. Prowse	7066	TSE spiking studies to assess the effectiveness of blood fractionation.	ends in 2002

All the above projects are reviewed on a regular basis by a steering group, chaired by Dr. T. Wallington.

P. Comer		Risk assessment of transmission by Blood	completed
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## Decontamination of surgical instruments

Lead Researcher	Contract number	Research subject	Status
D. Taylor	6691	Review of inactivation of TSEs.	Completed
C. Lowe	7021	Review of decontamination processes.	completed
N. Raven	7034	Use of an ultra-sensitive ELISA to detect prion proteins.	ends in 2002
R. Sommerville	7035	Removal of TSE agents by zeolite.	ends in 2002
K. Fernie	7041	Surgical instrument damage during decontamination.	report awaited
R. Sommerville	7042	Relative resistance to inactivation of different TSE strains.	ends in 2004
J. Collinge	7076	Study of implanted stainless steel wires to validate decontamination.	ends in 2002
C. Lowe J. Hope	7091/ 7117	Use of MARS to evaluate washing Procedures and surface modification.	ends in 2004,
D. Perrett	7112	Evaluation of various decontamination agents and the use of MALDI ToF MS to assess macromolecular residues.	ends in 2002
C. Lowe	7292	Use of MARS to detect prions on Glass and plastic surfaces	ends 2004
D. Perry	7389	Plasma sterilisation	under review
C. Keevil	007/0073	Development of rapid visual and epimicroscopy techniques	funding agreed
R. Baxter	007/077	Removal of infective protein residues from medical instruments	contract sent out
H. Reid	007/079	Creation of a validatable procedure to check decontamination protocols	contract sent out

All the above projects are reviewed on a regular basis by a steering group, chaired by Professor D. Jeffries.

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