



## **Risk of vCJD transmission via tissue transplantation: Work in progress**

### **ISSUE**

1. The Economics and Operational Research (EOR) division of the Department of Health (DH) and the National Blood Service (NBS) have produced a draft paper which set out an approach to assess the risks of vCJD transmission from tissue transplantation. A quantitative risk assessment was not considered feasible because of the scientific uncertainties surrounding the issue of infectivity in tissues.
2. This adds to the work already completed by EOR and the NBS, which includes an assessment of the risk of transmission of CJD from blood, surgical instruments, dentistry, and bone grafting.

### **Background**

1. There have been reported worldwide, around 300 cases of iatrogenic CJD cases (R G Will, 2003). Dura mater (n=136) and pituitary growth hormone (n=162) account for most of these cases. There have been three reported cases of iatrogenic transmission by ophthalmic tissue, one involving a corneal transplant in the US, and another two in Japan and Germany. The incubation period of the disease is determined by both titre of inoculum and site of inoculation. To date, the incubation period following grafts of dura maters ranges from 18 months to 18 years (median 6 years) and ranges from 5-30 years following injection of pituitary hormone (mean 12 years).
2. The assessment outlines the need to assess the risks of iatrogenic transmission via tissue transplantation. As with many of the issues involving transmissible spongiform encephalopathies (TSEs) there are scientific uncertainties involving the risk of infection. The assessment highlights these uncertainties and proposes a theoretical approach to assess the risk of iatrogenic infection from transplanted tissues.
3. The assessment aims to prioritise the order in which tissues are assessed and identifies areas where more research is required. There are two components to the suggested prioritisation process:
  - To conduct a systemic review of the expression of PrP<sup>Sc</sup> in the cell types of tissues and the epidemiology of iatrogenic human TSEs. This would be

considered in context of the clinical use of these tissues and available allogenic alternatives. A draft survey is provided in Annex A;

- Calculation of 'threshold' levels for tissue infectivity, which indicate the probability of transmission should a donor be infective with vCJD.
4. The UK blood services Standing Advisory Committee on Transfusion Transmitted Infections (SACTTI) have advised on the potential infectivity of tissues. Table 1 provides information on the tissue types used, the relative infectivity and other factors, which would effect the risk of CJD transmission. The relative infectivity of tissues provided in table 1 should be considered with data provided by the WHO guidelines (Annex B pages 14-18).
  5. Section 3 of the assessment provides two examples of the threshold calculations applied to corneal and sclera transplants. A threshold level of 2 ID<sub>50</sub> is used in a linear dose response model, where a transplant recipient receiving a dose of 2 ID<sub>50</sub> or higher would be infected. This threshold level of 2 ID<sub>50</sub> has been previously endorsed by the committee as a reasonable working assumption until experimental evidence becomes available.

## **ACTION**

Members are asked to consider if:

1. they endorse the suggested approach to assess the risk from different tissues.
2. Can the members suggest any alternative methodology?
3. Are members aware of any additional data relevant to tissue infectivity? Specifically do members consider that infectivity is likely to be proportional to mononuclear cell count (or to any other cell count)?

## **List of material attached**

1. [Annex 1](#) - SEAC 81/13 Draft 0.6 23rd January 2004. On the risk of vCJD transmission via tissue transplantation: Report on work in progress
2. [Annex A](#) - Notes on uses of tissues
3. [Annex B](#) - WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products

## ON THE RISK OF vCJD TRANSMISSION VIA TISSUE TRANSPLANTATION: REPORT ON WORK IN PROGRESS

Economics and Operational Research Division (EOR4)  
Department of Health  
80 London Rd  
London SE1 6LH

Draft 0.6: 23<sup>rd</sup> January 2004

### 1. Background and Purpose

- 1.1 While the number of people who might be incubating vCJD remains highly uncertain, a continuing concern has been to guard against the risk of secondary (i.e. person-to-person transmission). Much remains to be learnt about the mechanism of infection, but secondary transmission appears to require the prion protein PrP<sup>Sc</sup> present in those already infected to be transferred to - or possibly only to come into contact with – proteins within a another individual.
- 1.2 The main potential secondary routes of infection would therefore appear to be via blood or tissue donation, or by tissue remaining on surgical instruments that are then re-used. Precautions against such risks already include leucocyte depletion of donated blood, the sourcing of plasma products from outside the UK, efforts to improve the decontamination of surgical instruments and the use of single-use instruments. Previous EOR analyses have informed these and other measures.
- 1.3 Implants or transplants of tissue from one individual to another represent a further potential transmission route. As discussed further below, there is a single known instance of sporadic CJD (sCJD) being transmitted through a transplant of ocular tissue in the US, as well as one possible case in Japan and another in Germany. There have been over 114 cases of sCJD transmission from dura mater.<sup>1</sup> With vCJD, there is evidence of PrP<sup>Sc</sup> being widely dispersed through the body – e.g. in lymphoid tissue - rather than being confined to brain and eye as appears to be the case with sCJD<sup>2</sup>. As with other secondary transmission routes, the main concern is that infectivity may be present well before the onset of any symptoms of the disease (so that excluding donors with signs of neurological impairment would not remove the risk of transmission).

---

<sup>1</sup> Duffy P, Wolf J, Collins G, DeVoe AG, Streeten B, and Cowen D.:(1974): Letter: “Possible person-to-person transmission of Creutzfeldt-Jakob disease.” *N Engl J Med.*;290(12): pp 692-3.

Heckmann JG, Lang CJ, Petruch F, Druschky A, Erb C, Brown P, and Neundorfer B. (1997): “Transmission of Creutzfeldt-Jakob disease via a corneal transplant.” *J Neurol Neurosurg Psychiatry*, 63(3):pp 388-90.

Both the ocular and dura mater cases are reviewed in *Guidance from Industry US Department of Health and Human Services, FDA, CBER June 2002*

<sup>2</sup> Wadsworth JD, Hill AF, Beck JA, and Collinge J. (2003): “Molecular and clinical classification of human prion disease”. *Br Med Bull*; 66: pp 241-54.

- 1.4 Many different tissues are transplanted in the UK – some also being exported for transplant – for many different purposes. In many cases there is only very limited evidence as to whether the tissue might carry vCJD infectivity.<sup>3</sup> Existing lines of argument frequently rely on extrapolating evidence from animal models, often using other Transmissible Spongiform Encephalopathies (TSE diseases). A programme of research is currently under way to test tissues from vCJD patients, where samples can be obtained. As discussed below, however, the sensitivity of these tests means that negative results may not support strong conclusions about safety from vCJD risks, especially once the quantities of tissue transplanted are taken into account.
- 1.5 **This paper aims to set out a way forward.** Given these uncertainties involved, we do not believe that it will be feasible to complete a quantitative risk assessment in the near future. Nevertheless policy development needs to use such information as is available, in order to **prioritise attention to:**
- any additional precautions against vCJD transmission
  - areas for further scientific research, particularly on tissue infectivity
- 1.6 The suggested approach comprises two elements. One is systematically to **collate information** on relevant procedures, considering factors that would affect the magnitude of any vCJD risks – both to an individual recipient and within the population as a whole. (At the same time, we note additional factors that should be relevant to risk management - e.g. around clinical need, and the existence of any alternatives to allogenic tissue use.)
- 1.7 The second part of the approach is to carry out “threshold” calculations for tissue infectivity, showing the levels above which transmission from an infective donor would *either:*
- become highly-probable, *or*
  - reach some other “threshold” – e.g. the “1 in 100” risk of infection used by the CJD Incidents Panel in other contexts (e.g. receipt of implicated blood plasma derivatives).
- The latter threshold may provide some guidance to the Panel in determining whether recipients of tissue from vCJD-infected donors would be at sufficient risk to be placed in a “contactable” group. These calculations can then be related to the sensitivity of available tests, to investigate whether negative results could with confidence place the risk below one or both thresholds.
- 1.8 It may also be possible to estimate the potential infectivity of different tissue using theoretical arguments – e.g. based on the density of specific types of cell, and/or tissue ontogeny. However these are not pursued here, and would require substantial scientific input to develop.
- 1.9 **At this stage, we are seeking SEAC’s endorsement of the approach set out above, and inviting suggested improvements or alternatives.**

---

<sup>3</sup> World Health Organisation: *WHO Guidelines on TSEs in Relation to Biological and Pharmaceutical Products*. WHO, February 2003.

## 2. Information on Tissues and Procedures

- 2.1 In seeking information about tissue transplants, we have been able to draw on a small expert group to advise on key points as the work proceeded. [DN – names, affiliation of members to follow]. Particularly on the question of potential infectivity of tissues, we have also been able to draw on the CJD Working Group of SACTTI (the UK Blood Services Standing Advisory Committee on Transfusion-Transmitted Infections).
- 2.2 Table 1 below (Tissue Product Information) summarises some key information on various tissues from the point of view of possible vCJD risks. While not exhaustive, we believe that this does cover the tissues commonly used. Procedures involving use of the patient's own tissue or cells are irrelevant to the present discussion. Also omitted are other possible procedures involving use of tissue-engineered products, embryonic stem cells or cells from other stem cell lines. Our understanding is that so far these have only been used on a very limited scale, and in research settings where recipients are likely to have short life-expectancy. Nevertheless the discussion should be extended to cover these developments in due course.
- 2.3 For each of the tissues listed, the table gives summary information on factors relevant to risk assessment – and, potentially, to risk management. These are as follows:
- *Type of tissue* (e.g. vascular or non-vascular), which may be relevant to potential infectivity.
  - *Relative infectivity*. In the main, this column summarises the interim view of the CJD Subcommittee of SACTTI. The group did not feel able to provide numerical estimates (e.g. in ID<sub>50</sub> per gram or ml) for each tissue. However members suggested that potential infectivity could be roughly categorised into four levels, of which three appear in the table. These are “high” (comparable to CNS or posterior eye, of which there were no examples here), “medium”, “lower range” (comparable to blood, perhaps of the order of 2 ID<sub>50</sub> per ml<sup>4</sup>) and “low” (probably less than blood, and possibly zero).
  - *Tissue mass*: the amount of tissue typically transplanted or implanted. This acts as a multiplier with infectivity in determining the dose that would be transferred, and hence the risk of infecting the recipient.
  - *Site of transplant*: this is again relevant to individual risk, as the site may provide a more or less efficient route for inward transmission of vCJD. In line with previous SEAC advice, sites other than CNS and posterior eye are regarded as providing less efficient routes of transmission.

---

<sup>4</sup> The infectivity of human blood is itself subject to much uncertainty. A review of the literature (primarily on animal models) commissioned by the Department of Health and CJD Incidents Panel from risk consultants DNV suggested that *if blood is infective*, then a value of 2 ID<sub>50</sub> per ml would have some plausibility. This is used here as an illustrative scenario against which to compare the various tissues considered.

- *Any batching (or pooling) of donations* is noted in the next column. This records whether use is essentially “1 donor to 1 recipient” (which it is, in most rows) or whether each procedure involves tissue taken from more than one donor.
- *Numbers used per annum* records the estimated number of procedures carried out each year in the UK using the tissue in question.
- *Age of donors* records information in whatever form is available. This is of relevance to the extent that different age cohorts may have had differential exposure to the primary source of vCJD infection. The very young – especially those born from 1996 onward - and the old may have had less exposure. (However elderly donors may be more at risk of sporadic CJD, which might also be of concern with some tissues.)
- Finally, *age of recipients* again offers this information in whatever form is available. It is relevant both for the reason above, and because younger recipients infected with vCJD would generally have a greater chance of surviving long enough to suffer clinical symptoms of the disease. If so, they would also suffer a greater loss of life-years (or QALYs). In addition, longer-lived recipients would be more likely donate blood (if eligible) or to undergo surgery at some later point in life, increasing the risk of further onward transmission of the disease.

2.4 More detailed information on the usage of each tissue is being collected, primarily via the small expert group mentioned above. The current draft is summarised in Annex A. In addition to the categories listed above, information on *clinical need* is also noted and, where relevant, reasons for using allogenic procedures even if autografts (or other alternatives, such as mechanical heart valves) may be available. **Any further comment on the table or annex would be welcome.**

**Table 1: TISSUE PRODUCT INFORMATION**

	<b>Tissue type</b>	<b>Relative infectivity</b>	<b>Tissue Mass</b>	<b>Site of transplant</b>	<b>Batching (Donor : Recipient)</b>	<b>Numbers used (pa)</b>	<b>Age of Donors (yrs)</b>	<b>Age of Recipients (yrs)</b>
<b>Ocular Tissue - Cornea</b>	non vascular but living tissue	Mid range	8 to 12mm diameter buttons. 8mm button = 0.04g	Anterior eye	1:2 (2 corneas)	2500 corneas Median product size 8mm.	All ages – mean 62	Bimodal – median 24 & 75 yrs. Age matched to +/- 15yrs
<b>Ocular Tissue - Sclera</b>	vascular but stored in alcohol. White blood cells	Mid range – as Cornea	Scleral shell = 1.2g	Ocular surface, orbital implants, lid reconstruction. Near optic nerve.	1:2 (2 sclera)  1:1	200 whole sclera  plus some patch grafts	All ages – mean 62	All ages – some paediatric recipients
<b>Ocular Tissue – Limbal Stem cells</b>	vascular living cells/tissue	Mid range	Very small: up to 0.04g	Stem cells – corneoscleral limbus.	1: many in 25% of cases  1: 1 in 75% of cases	<50 stem cell grafts	All ages – mean 62	All ages
<b>Amniotic Membrane - Ophthalmic</b>	Non vascular	Lower range	5.2 mg/cm <sup>2</sup>	Ocular surface reconstruction	1: 40	157 of [2x2] cm 34 of [3x3] cm	30 (average)	All ages – mainly adults
<b>Amniotic Membrane - Vaginoplasty</b>	Non vascular	Lower range	5g – A5 size		1:1	Small number	30 (average)	Young adults
<b>Skin</b>	Vascular, processed  Dendritic cells	Lower range.	31 mg/cm <sup>2</sup>	External – Burns 10% with 2-3 appl'ns, 90% with 1 appl'n	1:1 2700cm <sup>2</sup> per donor, 2500 cm <sup>2</sup> per recipient	170000 cm <sup>2</sup> 500 pa (inc. 200 children) 0.33 m <sup>2</sup> per child, 0.9 m <sup>2</sup> per adult	48.5 (average)	All ages
<b>Peripheral blood stem cells (PBSC)</b>	Vascular	Lower range	30-300g 300-400ml leucocytes, 7-9L blood MT)	Venous infusion	1:1	To follow	2 - 60	0 - 65 with peaks for children and late middle aged
<b>Bone marrow</b>	Vascular	Lower range	100-1500g, 500ml-1L (MT)	Venous infusion	1:1	To follow	1 - 65	0 - 65 with peaks for children and late middle aged
<b>Placental cord blood*</b>	Vascular	Lower range	100-140ml (MT). Varies (see below)		1:1 normally, but some multiple units	12 transplants	0 (i.e. at birth)	median 8 yr, range 0.7 – 51

<b>DLI - Donor lymphocyte Infusions</b>	Vascular	Lower range	30-300g	Venous infusion	1:1	150 (in 2002)	16 - 60	0 – 65, mainly adults
<b>Heart valves</b>	Non vascular, acellular	Low, (as bone without blood & marrow)	Valves – 14.5 g, range 3.42-25.01g	Heart	1:1 or 1:2	7 - NBS 28 – SNBTS 595 – non BTS	0 - 61 median 44 mean 40	Children & Younger adults Aortic: 0-82 yrs (Md:14, Mean 24) Pulmonary: 0-64 (Md:8, Mean 12)
<b>Tendons</b>	Non vascular Processed, acellular	Low (as bone without blood & marrow)	70.4g per patellar tendon graft	Knee ligament repair	1:1	92 – NBS 20 – SNBTS from 6 donors	37 (average)	Younger adults
<b>Bone unprocessed**</b>	Bone (non vascular) + blood & marrow.	Blood & marrow -Lower range: Bone – low?	80g	Revision hip replacement.	1:1	2300	Older adults	Older adults
<b>Bone (processed)**</b>	Bone (non vascular) + residue blood & marrow.	Blood & marrow - Lower range Bone – low?	32g	Revision hip replacement.	May be 1:several (if donations are pooled)	2500	Older adults & cadaveric donors	Older adults
<b>Tissue engineered Products</b>	Not covered here							

\* See annex A for notes on unit volumes etc.

\*\* Potential vCJD risks from bone implants arise from any potential infectivity in bone itself, and from residual blood and marrow (reduced, but not completely eliminated by processing). See separate paper for detailed discussion of risks in different scenarios.

### 3. Discussion

- 3.1 Clearly, the potential risk of vCJD being transmitted via transfer of tissue is critically dependent on the relevant specific infectivities ( $ID_{50}$  per g or per ml). These are essentially unknown. The expert judgements summarised in Table 1 are clearly a helpful step in this direction, but stop short of full quantification (and were also offered on a tentative basis). For comparison, the relevant tables from the WHO Guidelines already referred to are reproduced at Annex B.
- 3.2 In terms of direct evidence of risk, there is no known case of vCJD having been transmitted by any of the procedures noted. It is difficult to draw firm conclusions even from the known transmission of sCJD by corneal transplant, given that the most sensitive methods available to date fail to reveal evidence of abnormal prion protein in the cornea of patients with either sCJD or vCJD. It is possible that the less refined microsurgical techniques of the 1970s may have played a part.
- 3.3 Firmer estimates of infectivity in different tissues could in principle come from:
- *Further animal studies* – albeit with the problems of extrapolating from experiments using other species, and often other TSEs.
  - *Experiments using tissues taken from known vCJD patients*. These are being undertaken as rapidly as possible subject to availability of tissues, but remain subject to limitations in sensitivity – e.g. the species barrier for human-into-mouse bioassay.
  - *Theoretical arguments* to support the presence of infectivity at different levels, for example based on:
    - counts of specific cell types (e.g.MNCs)
    - the presence of “normal” PrP in different tissues.
    - Ontogeny (origin in terms of stem cell lines) of the cells and tissues concerned.
- 3.4 At present, we are uncertain whether any of these lines of argument would command consensus among TSE researchers. This is something that we would welcome an opportunity to explore. However, these questions are not taken further here: to do so would require further and substantial scientific input.
- 3.5 For the present, a simpler approach is proposed. Rather than use a speculative estimate of the possible risk from each type of procedure, it may be more helpful to consider the converse question – **how low would infectivity (per g or per ml) have to be, for the total dose per operation to remain below some specified threshold?** This is relatively straightforward to answer, given the information gathered on the quantities of tissue transferred in each operation. This threshold calculation can then be related to any experimental evidence available, with particular attention to the levels of infectivity detectable by current methods. (It could also be related to the “theoretical” arguments noted above, if these could be further developed.)

- 3.6 To address this question, we need to specify the dose-response relationship, which defines the chance of an individual being infected by any given dose. The simplest possible model – and one endorsed by SEAC for vCJD in other contexts – is the *linear* dose response. This takes the probability of infection to be proportional to the dose received, up to a limit of 2 ID<sub>50</sub>. For doses above this, infection is regarded as certain.
- 3.7 Using this model, the dose of 2 ID<sub>50</sub> provides an obvious threshold to consider. Anyone receiving this dose, or higher, would be at very high risk of being infected. (“Certainty” of infection may be something of an overstatement for doses close to 2 ID<sub>50</sub>, as the linear model probably oversimplifies in this respect. But the risk of infection would certainly be high.)
- 3.8 Lower thresholds will also be of interest – in particular the dose of 0.02 ID<sub>50</sub>, which, on the simple linear model, would imply a **1 in 100** chance of transmission from an infective donor. This has been used in other contexts by the CJD Incidents Panel<sup>5</sup> to define the level of risk above which individuals would need to be contacted – and their exposure explained – to minimise the risk of further onward transmission.

#### 4. Examples of “threshold dose” calculations

- 4.1 The approach just outlined can most easily be explained by means of examples. To illustrate, we consider the first two rows of the “product table” above – i.e. corneal and scleral implants.

##### Corneal transplant

- 4.2 As shown in Table 1, this typically involves transferring approx 0.04g (40mg) of tissue.
- If the donor were infective, the threshold of 2 ID<sub>50</sub> per operation would thus be reached if specific infectivity is at least 50 ID<sub>50</sub> per gram.
  - However we have also to bear in mind that infectivity is specific to the route of transmission. Suppose that transmission into anterior eye is less efficient than inter-cranial by a factor of 10 (as used in the existing risk analysis for surgical transmission). Then reaching a per-operation dose of 2 ID<sub>50</sub> by this route would require corneal tissue to have a specific infectivity of at least **500 i/c ID<sub>50</sub>/g**.
  - Similarly, the “1 in 100” risk threshold would be reached with a specific infectivity of **5 i/c ID<sub>50</sub>/g**

---

<sup>5</sup> CJD Incidents Panel: *Management of Possible Exposure to CJD Through Medical Procedures: a Consultation Paper*, Dept of Health, October 2001

### Transplant of sclera

- 4.3 If it involves a whole sclera (rather than a smaller “patch”), this procedure typically transfers about 1.2 g of tissue.
- This implies that: “certain” transmission from an infective donor would be reached if the infectivity (for that route) exceeds 1.7 ID<sub>50</sub> / g.
  - Given the proximity of the implant to the optic nerve, it might be debated whether the 1-log “inefficiency” as compared with intra-cerebral transmission should apply. If it does not, then the 2 ID<sub>50</sub> dose would be reached at a tissue infectivity of **1.7 i/c ID<sub>50</sub> / g**.
  - Similarly, the “1 in 100” transmission risk would require an infectivity of at least **0.017 i/c ID<sub>50</sub> / g**

### Comparisons with Test Sensitivity

- 4.4 Ongoing research on human tissues by CJDSU (IN CONFIDENCE, yet to be published) has as yet found no evidence of infectivity in either cornea or sclera. Further work should clarify the sensitivity of the test used, but this *may* correspond to a detection level of the order of **500 i/c ID<sub>50</sub> / g**.
- 4.5 Taking this figure as an illustration, it would imply that
- For a corneal transplant, the maximum possible titre consistent with the evidence would give a dose exactly on the 2ID<sub>50</sub> (“certain infection”) threshold – and well above the 1-in-100 threshold used by the CJD Incidents Panel to determine “contactable” recipients. Though it must be remembered that this is a worst case, similar calculations have been used to inform the Panel’s view on – for example – recipients of plasma derivatives.
  - For a whole sclera, a level of tissue infectivity *well* below this detection limit would create a very high risk of transmission. All else being equal, this may therefore be a high priority for precautionary measures, particularly given that some recipients are young.

### Final Comments

- 4.6 These arguments can in principle be extended to the other tissues in Table 1. The results could also be refined to include the potential effects of any relevant processing steps – e.g. the option of gamma-irradiating items such as tendons.
- 4.7 The suggestion is offered with a caveat against using a 2 ID<sub>50</sub> threshold *too* literally. Not only is the linear dose-response model a simplification: in addition, higher doses might well result in shorter incubation periods – so it would still matter how far above the threshold a dose is. For the individual patient, receiving 200 rather than 2 ID<sub>50</sub>s would increase the chance of developing vCJD symptoms rather than dying of some other case first.
- 4.8 With this in mind, comments are invited as to whether this basic approach provides an appropriate way forward in a highly uncertain area.

## ANNEX A: NOTES ON USES OF TISSUES

*Note: information is as provided by (or via) members of expert group – some paraphrasing has been done for brevity.*

### **Ocular Tissue Transplants: Cornea and Sclera** (Andrew Tullo & George Galea)

Apart from limbal stem cell grafts (see below), ocular tissues are acquired from whole eyes removed from cadavers, usually within 24 hours of death. A serum sample is screened for Hepatitis B and C, HIV and syphilis. Following retrieval, the eyes are transported to one of several eye banks, where a corneoscleral disc (comprising the cornea and a narrow scleral rim) is excised from the anterior eye. Contents of the posterior eye are removed, leaving a scleral shell.

The disc is stored for up to 4 weeks prior to issue. A minority of corneoscleral rims are kept in cold storage at 4° for up to 10 days before transplantation.

In the cornea transplant unit, the central **cornea** is ‘punched out’ by the surgeon to replace a similar size button of diseased cornea in the recipient, and stitched into place. Corneas are avascular but are in close contact/ derived from neural tissue. The vast majority of recipients will only be exposed to 1 donor.

**Sclera** are cleaned and stored in alcohol for up to 6 months before issue for a variety of reconstructive procedures to deal with defects in the orbit, eyelid, or the eye globe itself.

### **Limbal Stem Cells** (Andrew Tullo)

The much rarer procedure of limbal stem cell transplant involves the surface of the junction between the edge of the cornea and sclera. This is the location of a ring of stem cells whose function is to replenish the surface cells of the cornea. Recent developments have led to the transplantation of small sections of this area to recipients with ocular surface stem cell failure. Most recently, efforts have been concentrated on *in vitro* expansion of very small numbers of donor cells from living related donors or from cadaver specimens which would allow several recipients to be in receipt of tissue from one donor.

Note: donors living / related to recipients?

### **Amniotic Membrane: Ophthalmic use** (Andrew Tullo, Ruth Warwick & Mr John Dart (AMTUG - amniotic membrane transplant user group))

This non-ocular tissue is now being used often as supplementary treatment to non-healing areas of the ocular surface, sometimes in conjunction with ocular tissue transplantation. The North London Tissue Bank is the main supplier and rarely has more than two donors in quarantine at any time as the membrane is large and is used to supply many small donor pieces (usually 2x2mm) which are placed on the ocular surface.

This application can be sight saving and also pain sparing. A clinical evaluation by AMTUG of ophthalmic indications for amnion is almost complete.

### **Amniotic Membrane: Vaginoplasty (Ruth Warwick)**

As with amnion for ophthalmic use, amniotic membrane can be donated by women at the time of a routine caesarean delivery. The placental membrane is removed and disinfected, then washed and the chorion peeled off. It is then sized, cut up, fixed onto nitro-cellulose paper and kept in glycerol. It is quarantined until after the donor is re-tested after 180 days. It is used in vaginoplasty to correct for radiation-induced or congenital defects for young pre-menopausal women. The first graft prepared by NBS was scheduled for transplant in November 03.

This type of gynaecological surgery is life-enhancing, allowing reconstructive surgery which might not otherwise be possible.

### **Skin (John Kearney & George Galea)**

Skin is generally grafted to cover large areas of exposed burnt skin or raw varicose ulcers. Generally, skin and these areas are highly vascular. Skin is used primarily for burns patients. As burns can occur at any age, the recipient population ranges from children to the elderly. There is no upper age limit for skin donors. Skin can be life-saving, particularly for large burns.

This is a temporary graft, used either to protect underlying autografts, or as a temporary closure of an excised wound. This allows time for previously harvested donor sites to heal, thus allowing a second harvest. Eventually the allograft is naturally sloughed from the wound or is deliberately removed by the surgeon.

Skin obviously has a large surface area to weight ratio. One feature of possible relevance to risk of disease transmission is that about 2% of the cells in the epidermis are dendritic antigen presenting cells (Langerhan's cells). Upon grafting, these cells process foreign antigens (from the recipient) and migrate to the recipient's lymph nodes. Their ability to carry prion protein needs expert opinion.

There is generally some processing - either disinfection or in some instances end sterilisation.

Mr Ken Dunn (President of the British Burns Association) has provided the following approximations on usage for adult and child patients. Their accuracy is limited by the lack of a national burns database.

There are 500 major burns cases per annum, about 200 of these being children. About 10% of these get 2-3 applications of cadaveric skin, about 5 days apart. The donors may be different in each case. The applications are about 50% of their surface area.

There are standard tables giving surface area measurements. Approximations from the tables at Great Ormond Street Hospital Paediatric Special care Unit suggest that:

If we presume an average child patient is 8 yr. old and weighs 15 Kg then the average Surface Area is 0.65 m<sup>2</sup>. For an average adult, say 65 Kg, the Surface Area is 1.8 m<sup>2</sup>

On average approximately 2700cm of skin is obtained from each donor. Skin has a mass of 31mg/cm<sup>2</sup>

**Haematopoietic stem cell (HSC)** (Marc Turner, Andrew Hadley & Ruth Warwick)

**[Peripheral Blood Stem Cells (PBSC),  
Bone Marrow Transplant (BMT)  
Placental Cord Blood]**

Haematopoietic stem cell (HSC) transplantation is used mainly for the treatment of patients with haematological malignancies (such as some forms of leukaemia and lymphoma), and occasionally in the setting of severely aplastic anaemia (bone marrow failure) and inherited metabolic or immunological defects (such as severe combined immune deficiency) in children. HSC are low frequency, self-renewing cells, mainly resident in the bone marrow, which give rise to the multiple different cell types of the haematopoietic (blood) and lymphoid (immune) systems. During the transplantation procedure the patient is subject to high dose chemotherapy and/or radiotherapy (the conditioning regimen), subsequent to which HSC are infused intravenously and home to the bone marrow where they engraft and generate a new haematopoietic and immune system. Allogeneic HSC transplantation (where the cells are derived from another individual) has a high procedure-related morbidity and mortality due to the toxicity associated with conditioning (particularly bone marrow failure) and the risk of the incoming immune system reacting against the patient's tissues if it sees them as "foreign" (termed: graft versus host disease).

A number of sources of HSC are in clinical use:

- Normal adult human peripheral blood contains HSC at such a low level that it would not be practicable to harvest these for therapeutic purposes.
- The original transplants were all harvested from bone marrow during which 500ml - 1.5 litre is aspirated from the donor's post-iliac crest under general anaesthetic.
- Over the last 10-15 years there has been increasing use of peripheral blood harvesting techniques. HSC are mobilised into the peripheral blood using 5 days of subcutaneous injections, followed by one or two apheresis procedures in which the donor's blood is passed through an extracorporeal circuit and the mononuclear cells separated by a centrifugation process in a continuous loop.
- Other products include donor leucocyte infusions which are used to try and re-establish chimerism or control recurrent disease following allogeneic bone marrow or PBSC transplant. These involve the original donor undergoing an apheresis procedure as described above but without the associated HSC mobilisation.
- Finally, cord blood derived from the placenta after birth can yield 44-240ml of blood, which is enriched in HSC. We do not know whether vertical transmission of variant CJD from mother to foetus occurs, and any risk from cord blood will obviously be dependent upon this. In the unlikely event that a mother develops variant CJD and the neonatal cord blood had been used for a transplant, we would have to try to decide whether this represented a risk of transmission, even though the child itself may not have evidence of variant CJD.

Note that in many cases the donor will be known to and closely related to the patient, the exceptions being matched unrelated donor transplants and cord blood transplants derived from registries/banks. Some of these will be donated in UK for patients in another country, and some UK transplants will be from foreign donors.

### **Donor Lymphocyte Infusions (DLI) (Andrew Hadley)**

The infusion of donor lymphocytes is of proven efficacy for some haematologic malignancies recurring after allogeneic stem cell transplant. Different illnesses vary in their responsiveness to donor lymphocyte infusions; chronic myeloid leukaemia is the most responsive, myeloma and low grade lymphoma less so. A new area of transplant biology is being developed, which uses virus-specific lymphocytes against infections such as cytomegalovirus. Expansion in vitro of lymphocytes that recognise tumour cells or viruses as well as specific depletion of lymphocytes that recognise normal recipient tissues is an ongoing area for study.

Donor lymphocytes can be prepared from peripheral blood, bone marrow, as a bi-product of CD34+ cell selection, or collected by apheresis. CD3 doses are aliquoted and cryopreserved until needed. Lymphocytes are often administered as a series of escalating doses ranging from around  $1 \times 10^6$  cells/Kg to over  $2 \times 10^8$  cells/Kg.

### **Heart Valves (Philip Yates)**

Abnormal function of the heart valves may be due to either the valve leaking when it should be tightly closed (regurgitation) or being narrowed and failing to open fully (stenosis). The cause may be congenital or acquired – e.g. following rheumatic heart disease. The increased strain on the heart muscle can compromise its long-term functioning as an effective pump, with signs of heart failure becoming evident. If left uncorrected, this may eventually result in premature death.

Treatments include drugs, surgical reconstruction, prosthetic heart valves and organ transplantation. Although routinely used, none are without constraints and complications.

Prosthetic heart valves have been used since the 1950's and may be mechanical or bioprosthetic, the latter being either:

- 'heterografts', composed of porcine or bovine tissue mounted on a metal support
- 'homografts' – i.e. preserved human heart valves, the main focus here.

Prosthetic heart valves differ from one another with regard to durability, thrombogenicity and effective orifice area.

- With rare exceptions, mechanical valves are very durable and last at least 20 to 30 years. In contrast 10-20% of homograft and 30% of heterograft prostheses fail within 10 to 15 years due to structural failure. Patients under 40 years of age have a particularly high incidence of premature heterograft failure.

- Thrombogenicity refers to the formation of a blood clot (thrombus) on the prosthetic valve. This may result in life-threatening disruption of valve function. Part of the thrombus may break off (embolise) and be carried in the blood stream down progressively smaller blood vessels until it eventually blocks a vessel. These emboli usually involve the blood supply to the central nervous system, and effects range from transient to fatal.
- To prevent these complications, life-long anticoagulation is recommended in all patients with mechanical valves and for the first few months with heterograft bioprosthetic. However, this introduces a risk of bleeding. Anticoagulation is not required for patients with homograft valves, a significant advantage.
- The homograft bioprosthesis has an effective orifice area similar to that of the native valve, superior to heterograft bioprosthesis and caged-ball mechanical valves of the same size.

Prosthetic-valve infection (endocarditis) occurs at some time in 3-6% of patients, either “early” (within 60 days after valve replacement) or “late”. Patients receiving mechanical valves or heterograft bioprostheses have a higher incidence of early endocarditis than those receiving homograft bioprostheses. This suggests a greater resistance by homograft bioprostheses to surface contamination acquired in the operating theatre. However later incidence is similar for all groups.

Overall, the most appropriate valve type will depend on balancing the various risk factors against the life expectancy and life style of the individual patient. For example bioprosthetic valves are preferred in patients who are elderly or have a life expectancy less than 15 years. Bioprosthetic valves are also preferred for patients who can not (or will not) take long term anticoagulation therapy, e.g. children or women who are pregnant (or planning to become pregnant), as warfarin anti-coagulation is contra-indicated in the first trimester of pregnancy.

Total non-BTS issues (Birmingham, Bristol, Oxford & Royal Brompton)

heart valves	595
conduits	40
patches	20

Some UK non-NBS valves are provided for non-UK patients in non-UK centres and to patients who come from abroad to have their operations in this country (although sometimes in the latter case non-UK valves are used). A significant proportion of heart valves from non-NBS banks is exported to Europe.

Data on mass is kindly supplied by Royal Brompton (Bob Parker) who issued 359 valves last year and weighed the valves on request as this is not routinely measured.

- For the 22 valves processed in the last week of July, the weight range was 3.42-25.01 gm (with a mean of 14.52 gm and standard deviation of 6.42)
- In 2002 the donor age range was 0-61 years (with a median of 44, mean of 39.21 and S.D. of 16.58)

- The recipients of aortic valves varied between 0-82 years (with median of 14, mean of 24.5 and S.D. of 24.12)
- The recipients of pulmonary valves varied between 0-64 years (with median of 8, mean of 12.15 and S.D. of 14.06)
- Donors of 0 means between 6 months and 1st birthday, Recipients of 0 between birth and 1st birthday (youngest was actually 2 days)

### **Tendons** (Philip Yates & John Kearney)

The most common uses for tendon allografts are for repair to injuries of the knee, rupture of the anterior cruciate ligament (ACL) being by far the most frequent. Untreated, this results in an unstable joints prone to recurrent episodes of 'giving way' with further damage to the joint and the early development of osteoarthritis.

The original injury can be caused by trauma (car crash) but also by sports injuries, skewing the recipient population towards younger adults. The treatment can be an effective means of maintaining knee stability and mobility and can allow the continuation of some sporting activities as well as preventing further damage and pain to the injured knee. The treatment is life- enhancing rather than life-saving.

In the past, acute primary repair of the ligament was a common treatment. However long term follow up studies reported deterioration with time, leading to the widespread belief that direct repair of the ACL is almost impossible.

A wide range of alternative types of procedure ("augmented repairs") has been investigated. These can be broadly classified into three groups.

1. prosthetic ligament - a permanent replacement for the normal ligament
2. stent - an internal splint device for temporarily protecting the acute primary repair or autologous graft
3. scaffold - providing a support for and stimulating ingrowth of host collagen tissue.

The best results to date have been obtained using homografts from either autologous or allogeneic (cadaveric) sources. One study showed that ACL reconstruction lowered the meniscus tear rate and need for subsequent surgery at two years from 27% to 3%.

### *Allografts versus autografts*

Various tissues from around the knee joint have been used for autografts. This is a mutilating surgery in which one structure is sacrificed to replace another, and complications are inherent. There is also increased morbidity associated with the harvesting of autologous tissue, with extensive surgical exposure, long tourniquet time and prolonged rehabilitation. The sacrifice of normal tissue in an already deficient knee may also increase functional disturbance over the longer term. Furthermore, the use of autografts limits not only the choice of tendon but also the amount of material available.

Tendon allografts have also been used for patients with chronic ankle instability, recurrent rupture of the Achilles tendon, and hand surgery. Allografts involve the use of cadaveric tissue in the form of patella tendon and Achilles tendon. Such grafts may illicit an immune response, although this is subclinical and markedly reduced following the deep freezing or freeze drying procedures whilst processing the tissue. Although autograft is still considered the gold standard for ACL reconstruction, a few clinical and experimental studies have shown no significant difference between allograft and autograft in terms of clinical outcome or biological remodelling of the graft. Some authors suggest a less predictable recovery with allograft because of a slower healing process and tendency to attenuate, but this has yet to be established by prospective controlled trials.

There are occasions when autografts are not available or undesirable, e.g.

- For revision surgery on patients in whom autologous tissue was used for previous reconstructive surgery which has now failed.
- For multiple ligament reconstruction (i.e. surgery in the dislocated knee), for which there is often insufficient autologous tissue
- For patients with unsuitable autologous tissue due to "small" size; previous trauma, degenerative joint disease or other reasons.

#### *Synthetic (prosthetic) alternatives*

A range of materials including dacron, polypropylene, PTFE, Gortex and carbon fibre have been used to manufacture synthetic ligaments. However there have been many clinical trials showing that these can neither provide a long-standing prosthetic substitute nor an innocuous scaffold. They are limited by not allowing adequate collagen ingrowth, and suffer from wear with first loosening and then eventually failure after about two years. They are also associated with sterile joint effusions. No long term studies of artificial ligaments support their routine use.

In summary, tendon allografts have significantly increased the treatment options for ligament injuries at a time when there is no suitable synthetic substitute. Whilst not universally recommended for all patients they are ideal in revision surgery, when autograft tissue is not available, and for athletes wishing to resume full sporting activity.

Generally the donor age (for both tendons & heart valves) is younger than bone donors. The upper age limit for tendon donors is 55.

The mass estimate shown for tendons in the "product table" (main text) is very high, due to being based on the patellar tendon graft. This graft has a bone plug on each end of the tendon, so really it is a composite graft of tendon together with bone.

Tendons like heart valves are both essentially organised collagenous tissues and do not contain significant amounts of marrow.

Tendons are inserted into joints mostly the knee and to replace like with like in other situations. These are relatively avascular areas.

**Bone**

Refer to separate analysis

**Note On Tissue Engineered Products (John Kearney)**

Though these are not considered in the present analysis, some information may be of interest for future reference.

At this stage the NBS intention is to produce tissue engineered grafts using human tissue as a matrix.

The plan is to use the same tissues as are currently banked, the difference being in the processing methodology.

1. The first step would be to remove all cells and cell components from the tissue matrix. Currently this is done using a combination of physical and chemical methods. The cells are lysed in a hypo osmotic buffer, and then treated with the detergent SDS to remove cell membranes and other components. The enzymes DNA'se and RNA'se are used to digest the nucleic acids.
2. The tissue matrix is thoroughly washed and may be sterilised (at present using peracetic acid).
3. The intention then is to harvest appropriate cells from the intended recipient and allow these to colonise the tissue matrix prior to implantation.

In most cases, NBS expect the cells to come from the recipient, i.e. autologous cells. The only allogeneic component would then be the decellularised tissue matrix. Thus the question here is whether de-cellularisation would decrease vCJD transmission risk.

However in certain cases an allogeneic cell line might be used. These would not be neurological or immunological cells, but rather specific tissue cells, e.g. skin fibroblast, heart valve fibroblasts, smooth muscle cells, etc. Once a cell line is established, these cells might be used to construct grafts for potentially hundreds or thousands of recipients.

**Members of ad hoc Expert Committee on vCJD and Tissues**

Elizabeth Love (chair): NBS

Andrew Tullo (Ophthalmic Surgeon, Manchester)

Philip Yates: SNBTS

Ken Dunn (President, British Burns Association)

Marc Turner: SNBTS (chair, SACCTI subgroup on vCJD)

John Kearney: NBS Tissue Services

Ruth Warwick: NBS Tissue Services

Derwood Pamphilon: NBS

Andrew Hadley: NBS

John Stephenson: DH (Research & Development):

Peter Doyle: DH (Tissues Policy)

Brett Jeffery: (SEAC secretariat)

Peter Bennett, Armin Kirthi-Singha: DH (EOR)

## **Annex B: Tables Extracted from WHO Guidance document on TSEs in relation to Biological and Pharmaceutical Products**

(Report of a WHO Consultation  
Geneva, Switzerland, 03-05 February 2003)

### **3.1.2 Tissue infectivity**

The foundation of any attempt to construct a rational analysis of TSE risk from biological and pharmaceutical products must begin with an evaluation of infectivity in the human or animal tissues from which these products are derived. Although straightforward in principle, the task is complicated by differences in the timing of first appearance and final tissue distribution of infectivity in different species and diseases, by differences in the sensitivity of bioassay methods, and by incomplete data about infectivity levels in various tissues of interest. Tables IA, IB and IC in Annex 1 summarize current data about the distribution of infectivity and PrP<sup>TSE</sup> in humans with variant CJD and other forms of TSE, in cattle with BSE, and in sheep and goats with scrapie. In general, it can be said that infectivity and/or PrP<sup>TSE</sup> has a wider distribution in vCJD than in non-variant forms of human TSE, and that infectivity in cattle with BSE has a much more limited tissue distribution than in any other studied variety of TSE, whether human or animal.

#### **Annex 1**

### **MAJOR CATEGORIES OF INFECTIVITY : TABLE IA, IB, IC**

The information in the Tables is based exclusively upon observations of naturally occurring disease, or primary experimental infection by the oral route (in cattle), and does

not include data on models using strains of TSE that have been adapted to experimental animals, because passaged strain phenotypes can differ significantly and unpredictably from those of naturally occurring disease. Because immunohistochemical and/or Western blot detection of misfolded host protein (PrP<sup>TSE</sup>) have proven to be a reliable indicator of infectivity, PrP<sup>TSE</sup> testing results have been presented in parallel with bioassay data. Tissues are grouped into three major infectivity categories, irrespective of the stage of disease:

- IA: High infectivity tissues: CNS tissues that attain a high titre of infectivity in the later stages of all TSEs, and certain tissues that are anatomically associated with the CNS.
- IB: Lower infectivity tissues: peripheral tissues that have tested positive for infectivity and/or PrP<sup>TSE</sup> in at least one form of TSE.
- IC: Tissues with no detectable infectivity: tissues that have been examined for infectivity and/or PrP<sup>TSE</sup> with negative results.

Although the category of lower risk tissues almost certainly includes some (e.g., blood) with a lower risk than others (e.g., lymphoreticular tissues), there are so few data about infectivity levels in these tissues that no attempt was made to subdivide the category into different levels of risk. It is also evident that the placement of a given tissue in one or another category can be disease specific, and subject to revision as new data accumulate.

Data entries are shown as follows:

- PrP<sup>TSE</sup> + Presence of infectivity or  
- Absence of detectable  
infectivity or PrP<sup>TSE</sup>  
NT Not tested  
NA Not applicable  
? Controversial or uncertain

results

( ) Data limited to one or two tested specimens (human tissues)

**Table IA: High infectivity tissues**

<b>CNS tissues that attain a high titre of infectivity in the later stages of all TSEs and certain tissues anatomically associated with the CNS</b>								
<b>Tissues</b>	<b>Human TSEs</b>				<b>Cattle</b>		<b>Sheep &amp; goats</b>	
	<b>vCJD</b>		<b>Other TSEs</b>		<b>BSE</b>		<b>scrapie</b>	
	<b>Infectivity<sup>1</sup></b>	<b>PrP<sup>TSE</sup></b>	<b>Infectivity<sup>1</sup></b>	<b>PrP<sup>TSE</sup></b>	<b>Infectivity<sup>1</sup></b>	<b>PrP<sup>TSE</sup></b>	<b>Infectivity<sup>1</sup></b>	<b>PrP<sup>TSE</sup></b>
Brain	+	+	+	+	+	+	+	+
Spinal cord	+	+	+	+	+	+	+	+
Retina, Optic nerve	+	+	+	+	+	NT	+	+
Spinal ganglia	NT	+	NT	+	+	+	+	+
Trigeminal ganglia	NT	+	NT	+	+	NT	NT	+
Pituitary gland <sup>2</sup>	NT	+	NT	+	-	NT	+	NT
Dura mater <sup>2</sup>	NT	-	NT	-	NT	NT	NT	NT

Annex 1

**Table IB: Lower infectivity tissues**

Peripheral tissues that have tested positive for infectivity and/or PrP <sup>TSE</sup> in at least one form of TSE								
Tissues	Human TSEs				Cattle		Sheep & goats	
	vCJD		Other TSEs		BSE		scrapie	
	Infectivity	PrP <sup>TSE</sup>	Infectivity	PrP <sup>TSE</sup>	Infectivity	PrP <sup>TSE</sup>	Infectivity	PrP <sup>TSE</sup>
<i>Peripheral Nervous system</i>								
Peripheral nerves	NT	+	(-)	+	-	NT	+	NT
Enteric plexuses <sup>3</sup>	NT	?	NT	(-)	NT	+	NT	+
<i>Lymphoreticular tissues</i>								
Spleen	+	+	+	-	-	-	+	+
Lymph nodes	+	+	+	-	-	-	+	+
Tonsil	+	+	NT	-	+	NT	+	+
Nictitating membrane	NA	NA	NA	NA	NT	-	NT	+
Thymus	NT	+	NT	-	-	NT	+	NT
<i>Alimentary tract</i>								
Oesophagus	NT	-	NT	-	-	NT	NT	+
Fore-stomach <sup>4</sup> (ruminants only)	NA	NA	NA	NA	-	NT	NT	+
Stomach/abomasum <sup>4</sup>	NT	-	NT	NT	-	NT	NT	+
Duodenum	NT	-	NT	NT	-	NT	NT	+
Jejunum <sup>5</sup>	NT	+	NT	-	-	NT	NT	+
Ileum <sup>5,6</sup>	NT	+	NT	-	+	+	+	+
Large intestine <sup>5</sup>	NT	+	NT	-	-	NT	+	+
<i>Reproductive tissues</i>								
Placenta <sup>7</sup>	NT	-	(+) ?	-	-	NT	+	+
<i>Other tissues</i>								
Lung	NT	-	+	-	-	NT	-	NT
Liver	NT	-	+	-	-	NT	+	NT
Kidney	NT	-	+	-	-	-	-	-
Adrenal	NT	+	-	-	NT	NT	+	NT
Pancreas	NT	-	NT	-	-	NT	+	NT
Bone marrow	NT	NT	(-)	-	+	NT	+	NT
Blood vessels	NT	+	NT	+	-	NT	NT	+
Olfactory mucosa	NT	NT	NT	+	-	NT	+	NT
Gingival tissue	NT	NT	-	-	NT	NT	NT	NT
Salivary gland	NT	NT	NT	NT	-	NT	+	NT
Cornea <sup>8</sup>	-	-	+	-	NT	NT	NT	NT
<i>Body fluids</i>								
CSF	NT	-	+	-	-	NT	+	NT
Blood <sup>9</sup>	-	-	-	-	-	NT	+	-

Annex 1

**Table IC: Tissues with no detected infectivity**

Tissues with no detected infectivity								
Tissues	Human TSEs				Cattle		Sheep & goats	
	vCJD		Other TSEs		BSE		Scrapie	
	Infectivity	PrP <sup>TSE</sup>	Infectivity	PrP <sup>TSE</sup>	Infectivity	PrP <sup>TSE</sup>	Infectivity	PrP <sup>TSE</sup>
<i>Reproductive tissues</i>								
Testis	NT	-	(-)	-	-	NT	-	NT
Prostate/ Epididymis/ Seminal vesicle	NT	-	(-)	-	-	NT	-	NT
Semen	NT	-	(-)	-	-	NT	-	NT
Ovary	NT	-	NT	-	-	NT	-	NT
Uterus (Non-gravid)	NT	-	NT	-	-	NT	-	NT
Placenta fluids	NT	NT	(-)	NT	-	NT	NT	NT
Foetus <sup>10</sup>	NT	NT	NT	NT	-	NT	-	NT
Embryos <sup>10</sup>	NT	NT	NT	NT	-	NT	?	NT
<i>Musculo-skeletal tissues</i>								
Bone	NT	NT	NT	NT	-	NT	NT	NT
Skeletal muscle <sup>11</sup>	NT	-	-	-	-	NT	-	NT
Tongue	NT	-	NT	-	-	NT	NT	NT
Heart/pericardium	NT	-	-	-	-	NT	-	NT
Tendon	NT	NT	NT	NT	-	NT	NT	NT
<i>Other tissues</i>								
Trachea	NT	-	NT	-	-	NT	NT	NT
Skin	NT	-	NT	-	-	NT	-	NT
Adipose tissue	NT	-	(-)	-	-	NT	NT	NT
Thyroid gland	NT	-	(-)	-	NT	NT	-	NT
<i>Body fluids, secretions and excretions</i>								
Milk <sup>12</sup>	NT	NT	(-)	NT	-	NT	-	NT
Colostrum <sup>13</sup>	NT	NT	(-) ?	NT	NT	NT	-	NT
Cord blood <sup>13</sup>	NT	NT	(-) ?	NT	-	NT	NT	NT
Saliva	NT	-	-	NT	NT	NT	-	NT
Sweat	NT	NT	-	NT	NT	NT	NT	NT
Tears	NT	NT	-	NT	NT	NT	NT	NT
Nasal mucus	NT	-	-	NT	NT	NT	NT	NT
Urine <sup>13,14</sup>	NT	NT	-	-	-	NT	NT	NT
Faeces	NT	NT	-	NT	-	NT	-	NT
Mammary gland/ udder	NT	NT	NT	NT	-	NT	-	NT

## Annex 1

### Footnotes

1. Infectivity bioassays of human tissues have been conducted in either primates or mice (or both); bioassays of cattle tissues have been conducted in either cattle or mice (or both); and most bioassays of sheep and/or goat tissues have been conducted only in mice (spinal cord, blood, and buffy coat have been bioassayed in sheep). In regard to sheep and goats not all results are consistent for both species.
2. No experimental data about infectivity in human pituitary gland or dura mater have been reported, but cadaveric dura mater patches, and growth hormone derived from cadaveric pituitaries have transmitted disease to scores of people and therefore must be included in the category of high-risk tissues.
3. In cattle, limited to the distal ileum.
4. Ruminant forestomachs (reticulum, rumen, and omasum) are widely consumed, as is the true stomach (abomasum). The abomasum of cattle (and sometimes sheep) is also a source of rennet.
5. In vCJD, positivity is limited to gut-associated lymphoid tissue (mucosa, muscle, and serosa are negative).
6. In cattle and sheep, only the distal ileum has been bioassayed for infectivity.
7. A single report of transmission of CJD infectivity from human placenta has never been confirmed and is considered improbable.
8. Because only one or two cases of CJD have been plausibly attributed to corneal transplants among hundreds of thousands of recipients, cornea is categorised as a lower-risk tissue; other anterior chamber tissues (lens, aqueous humor, iris, conjunctiva) have been tested with a negative result both in vCJD and other human TSEs, and there is no epidemiological evidence that they have been associated with iatrogenic disease transmission.
9. Early reports on the transmission of disease to rodents from the blood of patients with sporadic CJD have not been confirmed, and evaluation of the ensemble of experimental and epidemiological data relevant to TSE transmission through blood, blood components, and therapeutic plasma products fails to suggest transmission from blood of patients with any form of 'classical' TSE. Not enough data has accumulated to be able to make the same statement about blood from patients with variant CJD. Fetal calf blood contains no detectable infectivity, but in genotypically susceptible sheep with natural scrapie or experimentally induced BSE, transfusion of large blood volumes has transmitted disease to healthy sheep. Infectivity has also been demonstrated in studies of rodent-adapted strains of TSE.
10. Embryos from BSE-affected cattle have not transmitted disease to mice, but no infectivity measurements have been made on foetal calf tissues other than blood (negative mouse bioassay). Calves born of dams that received embryos from BSE-affected cattle have survived for observations periods of up to seven years, and examination of the brains of both the unaffected dams and their calves revealed no spongiform encephalopathy or PrP<sup>TSE</sup>.
11. Intracerebral inoculation of muscle homogenates has not transmitted disease to 1) primates from humans with sporadic CJD; 2) mice or cattle from cattle with BSE; and 3) mice from sheep and goats with natural or experimentally-induced scrapie. However, older reports described single instances of transmission from goat and hamster muscle, and a more recent report described transmission from the muscle of wild type and transgenic mice, but as each of these studies were conducted with passaged strains of TSE, their relevance to natural disease remains undetermined. A recent human case report described a patient with CJD and inclusion body myositis with abundant PrP<sup>TSE</sup> in diseased muscle. After much deliberation, the committee nevertheless elected to retain muscle in the 'no detected infectivity' tissue category until more information about uncomplicated natural infections becomes available.
12. Evidence that infectivity is not present in milk includes temporo-spatial epidemiologic observations failing to detect maternal transmission; clinical observations of over a hundred calves nursed by infected cows that have not developed BSE; and experimental observations that milk from infected cows has not transmitted disease when administered intracerebrally or orally to mice. Experiments are in progress in which large volumes of milk from experimentally infected cows are concentrated and tested for the presence of PrP<sup>TSE</sup>.
13. Single reports of transmission of CJD infectivity from human cord blood, colostrum, and urine have never been confirmed and are considered improbable.
14. A previously unreported PrP type, termed PrP<sup>u</sup>, has been identified in the urine of sporadic and familial CJD patients, but its significance for transmission risk remains to be determined.

## Annex 1

### REFERENCES

The Tables IA, IB and IC were created by an *ad hoc* expert group formed during the Consultation, the members of which were Dr R. Bradley; Dr P. Brown; Prof. Dr H. Budka; Prof. Dr D. Dormont; Dr M. Groschup; Dr B.E.C. Schreuder; Dr G.A.H. Wells. Dr P. Brown consolidated the data and information provided after the Consultation.

Most of the observations that form the basis for the Table have been published in original reports (or cited in reviews) that follow. No attempt has been made to list the many reports in which only one or two tissues were examined, unless they concerned tissues of exceptional current interest. Also, a number of observations made by, or known to, members of the expert subcommittee that assembled the table, have not yet been published.

#### Human TSE

Brown P, Gibbs CJ Jr, Rodgers-Johnson P, Asher DM, Sulima MP, Bacote A, Goldfarb LG, Gajdusek DC. Human spongiform encephalopathy: the National Institutes of Health Series of 300 cases of experimentally transmitted disease. *Ann Neurol* 1994 35: 513-529

Bruce ME, McConnell I, Will RG, Ironside JW. Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. *Lancet* 2001; 358: 208-209

Hainfellner JA, Budka H. Disease associated prion protein may deposit in the peripheral nervous system in human transmissible spongiform encephalopathies. *Acta Neuropathol* 1999; 98: 458-460

Head MW, Northcott V, Rennison K, Ritchie D, McCardle L, Bunn TJ, McLennan NF, Ironside JW, Tullo AB, Bonshek RE. Prion protein accumulation in eyes of patients with sporadic and variant Creutzfeldt-Jakob disease. *Invest Ophthalmol Vis Sci* 2003; 44: 342-6

Ironside JW, McCardle L, Horsburgh A, Lim Z, Head MW. Pathological diagnosis of variant Creutzfeldt-Jakob disease. *Acta Pathol Microbiol Immunol Scand (APMIS)* 2002; 110: 79-87

Wadsworth JDF, Joiner S, Hill AF, Campbell TA, Desbruslais, M, Luthert PJ, Collinge J. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet* 2001; 358: 171-180

Wong BS, Green AJ, Li R, Xie Z, Pan T, Liu T, Chen SG, Gambetti P, Sy MS. Absence of protease-resistant prion protein in the cerebrospinal fluid of Creutzfeldt-Jakob disease. *J Pathol* 2001; 194: 9-14

#### Bovine Spongiform Encephalopathy

Houston F, Foster J D, Chong A, Hunter N, Bostock CJ. Transmission of BSE by blood transfusion in sheep. *Lancet* 2000; 356: 999-1000

Update of the Opinion on TSE Infectivity Distribution in Ruminant Tissues. Scientific Steering Committee of the European Commission for Food Safety. Internet address: [http://europa.eu.int/comm/food/fs/sc/ssc/outcome\\_en.html](http://europa.eu.int/comm/food/fs/sc/ssc/outcome_en.html)

Wells GAH, Hawkins SAC, Green RB, Austin AR, Dexter L, Spencer YI, Chaplin MJ, Stack MJ, Dawson M. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Vet Rec* 1998; 142: 103-106

## Annex 1

### Scrapie

Hadlow WJ, Kennedy RC, Race RE. Natural infection of Suffolk sheep with scrapie virus. *J Infect Dis* 1982; 146: 657-664

Hadlow WJ, Kennedy RC, Race RE, Eklund CM. Virologic and neurohistologic findings dairy goats affected with natural scrapie. *Vet Pathol* 1980; 17: 187-199

*Houston F et al. Lancet 2000;356:999-1000*

Hunter N, Foster J, Chong A, McCutcheon S, Parnham D, Eaton S, MacKenzie C, Houston F. Transmission of prion diseases by blood transfusion. *J Gen Virol* 2002; 83: 2897-2905

Race R, Jenny A, Sutton D. Scrapie infectivity and proteinase K-resistant protein in sheep placenta, brain, spleen, and lymph node: implications for transmission and antemortem diagnosis. *J Infect Dis* 1998; 178: 949-953

Van Keulen LJM, Schreuder BEC, Vromans MEW, Langeveld JPM, Smits MA. Pathogenesis of natural scrapie in sheep. *Arch Virol* 2000; 16 (Suppl): 57-71