APPENDIX II

INFECTIVITY OF BLOOD

Contents

II. INFECTIVITY OF BLOOD

II.1 Introduction

II.1.1 Outline of Appendix

The purpose of this appendix is to estimate the level of infectivity in blood from donors infected with new variant CJD, and the proportions of infectivity that might remain in the various blood products. The appendix gives a brief outline of CJD, and reviews the available evidence on the level of infectivity in blood and blood components. From the little quantitative information that is available, it makes initial estimates of infectivity in blood.

II.1.2 Units of Measurement

Infectivity is measured in this study in infectious dose units (ID_{50}), defined as the dose that would cause infection of 50% of the exposed population. Infectivity concentration in blood products is measured in terms of ID_{50} per ml of product. Annual risks from infectivity will be measured in terms of ID_{50} per year. The relevant pathway for infection is via blood transfusion or blood product injection into humans. Hence, infectivity is always measured in human intravenous (i/v) ID_{50} units, unless otherwise stated.

II.2 Introduction to CJD

II.2.1 TSEs

CJD, BSE and scrapie are all varieties of transmissible spongiform encephalopathy (TSE) occurring in humans, cattle and sheep respectively. The distinctive feature of all TSEs is the development of sponge-like holes in brain tissue, resulting in a deteriorating mental condition and eventually death. The diseases develop slowly without external symptoms for several years, and death typically follows soon after the onset of clinical symptoms. The diseases can be transmitted from one person to another, and even from one species to another, if sufficient infected tissues are transplanted, inoculated or eaten.

Despite extensive research, there are still many gaps in our knowledge about the nature of TSEs. Experimental research takes a long time to produce results because the diseases themselves develop so slowly. Most research concerns scrapie in laboratory mice, and it is unclear how far its conclusions might apply to TSEs in other species. Theoretical research is difficult because of lack of knowledge about the infective agent. Epidemiological studies are difficult because of the long incubation period, and the fact that there is at present no way to test for the disease except by removing a sample of infected tissue, which cannot normally be done until the victim is dead.

As a result, no one knows how many people may be incubating the disease already, and unless a simple test is developed this knowledge is unlikely to be gained until the majority of those infected are already dead or dying. This makes it extremely difficult to develop a public health policy to minimise further infections and to care for those already infected.

II.2.2 The Infective Agent

The nature of the agent that causes infection with TSEs is unclear. It is unlike a conventional virus or bacteria, as it stimulates no immune response in the host, and is resistant to inactivation by heat, chemical disinfection or radiation.

The dominant theory is that the agent is an abnormal form of prion protein (PrP). In healthy animals, prion is present in many organs and tissues, including the brain. Its function is unknown. When an animal is infected with TSE, the abnormal form of PrP progressively accumulates. It is not clear whether this is the cause of the symptoms, or a result of some other unknown cause. The prion theory assumes that abnormal prion is able to induce neighbouring prion to distort similarly. In this way, the infection is able to spread slowly through the body, following a chain of prion-expressing tissues to the brain.

According to this theory, there is little genetic involvement in the disease, as the prion protein distorts after the molecules have been formed. The gene encoding prion protein has been identified, and it has been possible to develop laboratory mice that do not have it, and these are completely resistant to inoculations of scrapie.

There are several other theories about the nature of the infective agent, as the concept of a replicating protein containing no nucleic acid is not universally accepted. One theory is that it is an unconventional virus, resistant to conventional methods of inactivation. It is possible

that other agents, producing similar effects, would lead to risks similar to those estimated using the prion theory.

II.2.3 The Development of the Disease

Tests with mouse-adapted scrapie have revealed that intracerebral inoculation directly into the brain establishes infection there directly, although symptoms may not appear for several months or even years. Once spongiform damage has reached certain critical areas of the brain, clinical symptoms become apparent. The infection also radiates into the peripheral nervous system. Following intracerebral inoculation, infectivity also enters the blood stream and lympho-reticular system, as for peripheral inoculation (see below).

Peripheral inoculation (e.g. intravenous) causes infectivity to be dispersed widely and rapidly in the blood stream, but it is removed in a few hours, possibly by the spleen. There follows a period in which the inoculum can be detected but there is no apparent replication of infectivity. Detectable replication of infectivity begins in the lympho-reticular system (particularly the spleen and lymph nodes). Its route from the spleen to the brain is not known, but is assumed to be via nerve fibres and the spinal cord. Once established in the spinal cord, infectivity spreads slowly to the brain. The result is then similar to intracerebral inoculation, but requires a greater quantity of inoculated tissue and follows a much longer incubation period.

Work (Klein et al 1997) showed that B lymphocytes (or B cells, a form of white blood cell involved in the immune response) may be necessary for the disease to spread to the brain. The precise role of B cells in TSEs is unknown, but it is possible that they transfer the infection into nerve cells within the spleen, and also that they carry it through the blood stream, accounting for its wide-spread presence in the organs of the lympho-reticular system. However, it is not necessarily the case that the B cells are carrying the infectious agent.

Other work has implicated follicular dendritic cells (FDCs) in the spreading of the disease to the brain. FDCs are cells of the lymphatic system that form the germinal centres of lymph nodes. They trap antigens and present them to the lymphocytes. Since B cells are required for maturation of FDCs, the two cell types are closely correlated. Work by Klein et al (1997) discounts FDCs from the spreading of disease to the brain, but this is disputed in other work (unpublished).

Following experimental intragastric infection of a cow (e.g. after eating infected tissue), the first organ in which infectivity is detected is the lymph node (Peyer's patches) in the wall of the small intestine. Although the infectious agent persists in this organ for some time, it has not yet been established whether it replicates there, and hence whether the Peyer's patch take the role of the spleen in the development described above. The result is then similar to peripheral inoculation, but is thought to require a greater quantity of tissue to cause infection and to follow a still longer incubation period.

II.2.4 CJD

Creutzfeldt-Jakob disease (CJD) is a type of TSE occurring in humans. It occurs mainly among people aged 55-80. It produces a deteriorating mental condition, involving loss of

memory and mental faculties, typically leading to death within 6 months of the onset of symptoms. At present, the infection is impossible to detect before symptoms develop; the disease is difficult to confirm before death occurs; and there is no known cure. There are approximately 50 cases of CJD in the UK per year.

CJD is known to be transmissible if infected tissues from someone with the disease are implanted in another person. This iatrogenic transmission has been documented where tissues used in medical or surgical procedures (e.g. human growth hormone treatment, corneal transplants or brain surgery) have later been found to be from donors incubating CJD. However, very few of the CJD cases (less than 1%) result from this form of transmission.

Most cases of CJD (about 85%) are sporadic, with no known cause, possibly due to the random change of prion within the victim. Genetic variations may give some degree of resistance to the disease, as some genotypes are more likely to suffer the disease. Some cases (about 14%) are familial, associated with mutations in the PrP gene.

There is no evidence that scrapie or BSE are the cause of the majority of CJD cases. CJD occurs at the rate of approximately 1 case per million people per year world-wide. It occurs in Australia and New Zealand, where there is no scrapie or BSE, at the same rate as in other countries.

II.2.5 BSE

Bovine spongiform encephalopathy (BSE) is a type of TSE occurring in cattle. It was first identified in 1986. Most known cases of the disease have occurred in Great Britain, although some cases have occurred in other parts of Europe, possibly due to export of infected animals or feed. The number of cases reached a peak in 1992-93, and has since declined very significantly.

The main source of the infection appears to have been via meat and bone meal (MBM), which was used to provide protein in cattle feed. MBM is obtained by rendering residues from carcasses of animals, including both cattle and sheep. A change in the method of rendering, which occurred mainly in England in the early 1980s, may have allowed prion protein from scrapie-infected sheep brains to enter the cattle food chain. Once established, rendering of cattle-brains may have recycled the infectivity. This theory is supported by the fact that the ban on feeding ruminant-derived protein to ruminants, which was introduced in 1988, was followed 5 years later (the typical incubation period) by a decline in the number of BSE cases.

II.2.6 Variant CJD

Between 1994- , XX cases of CJD have been identified in the UK which differ from the normal type of CJD. They occur in younger patients (aged 16-53), produce different symptoms, and have a different pattern of lesions in the brain. This variation is referred to as variant Creutzfeldt-Jakob disease (vCJD). Characteristic aspects of vCJD are so similar to those of BSE and different to sporadic CJD and other TSEs that a common agent must be the cause.

The most likely cause of vCJD is consumption of beef products infected with offal before it was banned from human food in 1989. Most vCJD cases occurred in the UK (there was one in France). All the cases had eaten beef or beef products in the last 12 years, but none had eaten brain. One had eaten no meat for 11 years; another for 3 years.

The vCJD cases appear to be occurring at a rate of about 10 to 20 per year. It is not yet possible to predict from them how many people might be infected with vCJD. Estimates from risk analysis are highly uncertain.

II.3 Infectivity in Blood

II.3.1 Experimental Evidence for Infectivity

II.3.1.1 Tests on Blood from Human CJD and vCJD Cases

Available experimental evidence on the infectivity of human blood from CJD and vCJD victims is summarised in Table II.3.1 (from a review by Brown 1995). All these experiments involved inoculating the blood into animals. If the animal died, its brain was then examined for signs of TSE.

Table II.3.1 Attempts to detect Infectivity in the Blood of Humans with CJD

Study	Donor Diagnosis	Material Inoculated	Assay Animal	Route	Transmissions/ Total Inocul'd*
Manuelidis et al (1985)	Sporadic CJD	Buffy coat	Guinea pig Hamster	i/c	2/2
Tateishi (1985)	Sporadic CJD	Whole blood	Mouse	i/c	1/3
Tamai et al (1992)	Sporadic CJD	Concentrated plasma	Mouse	i/c	1/1
Brown et al (1994)	Sporadic CJD	Whole blood	Chimpanzee	i/v	0/3
	Sporadic CJD	Whole blood	Spider monkey	i/c, i/v, i/p	0/1
	Sporadic CJD	Whole blood	Squirrel monkey	i/c, i/p, i/m	0/1
	Sporadic CJD	Buffy coat	Squirrel monkey	i/c, i/p	0/4
	Sporadic CJD	Whole blood	Guinea pig	i/c, i/p	0/1
	Kuru	Serum	Mouse	i/c	0/3
Deslys et al (1994)	hGH CJD	Whole blood	Hamster	i/c	1/1
Bruce et al (2001)	vCJD	Buffy Coat	Mouse	i/c	0/18
		Plasma	Mouse	i/c	0/23

* Fraction of blood samples found to transmit disease. Number of animals infected is larger.

The table shows that bioassays on mice failed to detect infectivity in blood components (buffy coat and plasma) from vCJD cases. In 4 experiments infectivity was detected in the recipient animal (mouse, hamster or guinea pig). Three used blood from sporadic CJD cases, and one used the blood of an iatrogenic CJD case. However, several other studies have failed to show infectivity in the blood of sporadic CJD cases when inoculated into monkeys, chimpanzees and guinea pigs. These conclusions are surprising because it would be expected that primates would be relatively easy to infect with human CJD, and rodents would be relatively difficult. A possible explanation is that blood is infective, but only at a level near the detectability threshold in the tests, at least when crossing the species barrier. Another possible explanation is that all the experiments that showed infectivity were flawed. Although this seems unlikely for 4 independent experiments, they all involved very few animals and all had some puzzling features (Brown 1995).

Hence these studies are taken to indicate the *possibility* of infectivity in the blood of human victims of sporadic and iatrogenic CJD, and they do not amount to proof. In the absence of better data, it would be appropriate to assume that the blood of sporadic and iatrogenic CJD cases is infective at a low level.

II.3.1.2 Tests on Blood from Animals with TSEs

Available experimental evidence on the infectivity of blood from animals with TSEs is summarised in Table II.3.2 (based on a review by Brown 1995). These tests have the advantage that inoculation does not have to be across a species barrier, but they have the disadvantage that human CJD may differ from experimental (e.g. mouse-adapted) CJD and scrapie, and hence the results may be invalid for humans.

Table II.3.2 Attempts to detect Infectivity in the Blood of Animals with TSEs

Study	Host Animal	Material Inoculated	Assay Animal	Route	Transmissions/ Total Inocul'd*
Natural scrapie					
Hadlow et al (1980)	Goat	Clot/serum	Mouse	i/c	0/3
Hadlow et al (1982)	Sheep	Clot/serum	Mouse	i/c	0/18
Experimental scrapie					
Pattison & Millson (1962)	Goat	Whole blood	Goat	i/c	0/14
Gibbs et al (1965)	Sheep	Serum	Mouse	i/c	1/1
Clarke & Haig (1967)	Rat	Serum	Rat	i/c	1/1
Clarke & Haig (1967)	Mouse	Serum	Mouse	i/c	1/1
Eklund et al (1967)	Mouse	Whole blood	Mouse	i/c	0/39
Dickinson et al (1969)	Mouse	Whole blood	Mouse	i/c	3/13
Hadlow (1974)	Goat	Clot	Mouse	i/c	0/3
Diringer (1984)	Hamster	Blood	Hamster	i/c	5/5
Casaccia et al (1989)	Hamster	Blood	Hamster	i/c	10/11
Experimental CJD					
Manuelidis (1978)	Guinea pig	Buffy coat	Guinea pig	All	10/28
Kuroda (1983)	Mouse	Buffy coat	Mouse	i/p	4/7
Brown et al (1998)	Mouse	All components	Mouse	i/c	
Brown et al (1999)	Mouse	All components	Mouse	i/c	
Experimental BSE					
Taylor et al (2000)	Mouse	Plasma	Mouse	i/c	4/48
Houston et al (2000)	Sheep	Whole blood	Sheep	i/v	**

Fraction of blood samples found to transmit disease. Number of animals infected is larger.
** Only preliminary data available

The table shows that infectivity has been found in the blood of laboratory mice, sheep and guinea pigs infected with CJD, when inoculated back into the same species. Infectivity has also been found in the blood of laboratory sheep, mice, rats and hamsters infected with scrapie. This again suggests that the level of infectivity in blood is near the detectability threshold in the tests.

The studies that showed infectivity mainly involved direct intracerebral inoculation of infected blood. Some peripheral inoculations of infected blood have caused infections, but intravenous transfusion of blood from human CJD cases into chimpanzees did not lead to infection. This would be expected if the intracerebral infectivity was near the detectability threshold, as peripheral inoculation is less efficient in most experimental models. However, transfers within the same species are more efficient, and preliminary reporting (Houston et al 2000) indicates that transfusion of whole blood (not leucodepleted) from a BSE infected but asymptomatic sheep to an uninfected sheep has resulted in transmission of the BSE infection.

The studies of experimental TSE demonstrate that blood is infective, at least in some strains of TSEs. They provide further evidence for the *possibility* of infectivity in the blood of human CJD victims, particularly vCJD, which has similarities to experimental CJD in animals (see Section II.3.3). In the absence of better data, it would be appropriate to assume that the blood of vCJD cases is infective at the same level as animals with experimental CJD.

II.3.2 Epidemiological Evidence for Infectivity

Several epidemiological studies have reviewed CJD cases to investigate whether they may have resulted from blood transfusion. Epidemiological studies in the UK, the USA and Japan (reviewed by Ricketts et al 1997) have shown that CJD victims have no more than average exposure to blood transfusions. This suggests that blood transfusion is not a major cause of infection for sporadic CJD cases.

The Annual Report 2000 from the National CJD Surveillance Unit reports a case control study of vCJD risk factors among 25 cases and 53 community controls. Three of the cases were reported by relatives to have had a history of blood transfusion compared with 3 of the 53 community controls. Although this suggests that previous blood transfusion may have been the cause in one or two of the 25 vCJD cases, there is a 30% likelihood that this result arose by chance due to the relatively small numbers involved. This information leaves open the possibility that blood transfusion may be a factor in vCJD transmission.

Four Australian CJD cases were identified who had received blood transfusions (Klein & Dumble 1993). Their symptoms were consistent with those of iatrogenic CJD, but there is no clear evidence that blood transfusions were the cause.

A Canadian CJD case had received albumin from a pool that included the blood of a CJD victim (reported by Ricketts et al 1997). However, the recipient died only 8 months after receiving the albumin, which short incubation period suggests this was not the cause of infection.

A similar French CJD case received albumin during a liver transplant from a pool that included the blood of a probable CJD victim (Creange et al 1996). The donor developed probable CJD 3 years after the donation. The recipient died only 2 years after receiving the transfusions, which is an unusually low incubation period, and suggests that the albumin was not the cause of infection.

Other studies have reviewed recipients of blood products to investigate whether they may be at increased risk of developing CJD. A review of haemophilia centres in the USA revealed no CJD cases in haemophiliacs (reported by Ricketts et al 1997).

It is understood (letter from National Blood Authority to CJD Incidents Panel 2001) that at present there are no reported cases of vCJD among the haemophiliacs and immunodeficient patients who have been exposed during regular replacement therapy to potentially contaminated UK plasma derivatives (factor VIII and IV/Ig G) by vCJD. This may be explained however by a long incubation period.

Investigation of a CJD victim who had donated 35 units of blood in 20 years identified 27 people who had definitely received the patient's blood, and 8 probable recipients (Schlesselmann 1982). For 20 units the recipients could not be identified. Of the 35 identified recipients, 18 had died (51%). None of the identified recipients had neurological disease.

A similar study of a German CJD victim who had donated 55 units of blood (Heye et al 1994) identified 35 recipients, of whom 21 (60%) had died from non-CJD illnesses up to 22 years later, and 14 were alive an average of 12 years after receiving the blood.

In an ongoing US study of patients who received blood from CJD victims (reported by Ricketts et al 1997), 147 recipients have been identified, of whom 80 have died (54%). There were no CJD cases among the 65 for whom the cause of death is known.

A retrospective surveillance study for CJD among persons with haemophilia has been reported by Evatt et al (1998). Samples from 24 haemophilia decedents were obtained and examined for signs of spongiform encephalopathy by a panel of expert neuropathologists. The panel found no evidence of CJD in any of the specimens.

It is possible that cases of transmission of CJD might be masked by the much larger numbers of sporadic cases. However, this would only apply to older recipients, since there are significant transfusions to neonates, and cases of CJD in young people are rare. This could not explain the recent incidence of vCJD in young people because similar levels of sporadic CJD (and hence infectivity in blood) occur in all countries.

It is concluded from the above that there is no evidence that sporadic CJD has ever been transmitted by blood transfusion. Although such transmissions may have occurred, the numbers would have to have been very small to escape detection. However, it is not certain that this also applies to vCJD.

II.3.3 Theoretical Evidence for Infectivity

Most of the evidence above applies to sporadic CJD in humans or to experimental scrapie and CJD in animals. There are some theoretical reasons (with limited experimental support) why infectivity may be more likely or higher in the blood of vCJD cases:

- vCJD is an *acquired* disease, which may develop in peripheral tissues and possibly circulate in the blood in a manner different to sporadic CJD. The available experimental evidence is consistent with a difference of this type. The one animal in one experiment on an iatrogenic CJD case (Table II.3.1) showed infectivity in the blood, whereas experiments with sporadic CJD only irregularly showed this. Experiments on CJD in animals (Table II.3.2), which is also an acquired disease, all showed infectivity in the blood, whereas experiments with scrapie only irregularly showed this. This suggests that infectivity in the blood may be higher in vCJD than sporadic CJD.
- Preliminary results (Houston et al 2000) indicate that transfusion of whole blood (not leucodepleted) within the same species from a sheep (asymptomatic) experimentally infected with BSE to another has resulted in the transmission of the BSE infection.

It is concluded from the above that infectivity in the blood may be more likely or higher in vCJD cases than in sporadic CJD. Hence the epidemiological evidence against infection via blood may be valid for sporadic CJD but not for vCJD.

II.3.4 The Variation of Infectivity in Blood Through the Incubation Period

II.3.4.1 Tests by Manuelidis et al

Manuelidis et al (1978) investigated viremia (infectivity in blood during the incubation period) in experimental CJD in hamster-adapted CJD from intracerebral inoculation. The results were expressed in terms of numbers of recipient hamsters with CJD, but were incomplete when reported and difficult to interpret. Nevertheless, they indicate the presence of infectivity throughout the incubation period.

II.3.4.2 Tests by Kuroda et al

Kuroda et al (1983) investigated the variation of infectivity through the incubation period in mouse-adapted CJD from intracerebral inoculation. The results were expressed in terms of numbers of recipient mice infected and their mean incubation period. The results for blood are given in Table II.3.3. They show significant infectivity in the blood starting about 30% of the incubation period and increasing towards the end of the incubation period.

Table II.3.3 Infectivity in Blood by Kuroda et al

Time After Infection Of Donor (Assay) Animal (Weeks)	Recipient Animals Infected	Mean Incubation Period Of Recipient Animal (Days)
1	0/4	-
2	0/4	-
3	0/2	-
6	5/5	281
9	3/3	213
14	3/3	156
18	5/5	142

II.3.4.3 Tests by Diringer

Diringer (1984) estimated the infectivity in the blood from donor hamsters with hamster-adapted 263K scrapie from intraperitoneal inoculation, early in the incubation period. The blood was concentrated so that each 50 µl of inoculum contained 2 ml blood equivalent. This was inoculated intracerebrally into groups of recipient hamsters. Table II.3.4 gives the results. It indicates a relatively high level of infectivity up to 40 days, i.e. about the first third of the incubation period. It gives no information on the later period.

Table II.3.4 Infectivity in Blood by Diringer

Time After Infection Of Donor (Assay) Animal (Days)	Recipient Animals Infected	Mean Incubation Period Of Recipient Animal (Days)
5	1/6	214
10	8/8	151
20	3/3	186
30	4/4	191
40	2/2	150

II.3.4.4 Tests by Casaccia et al

Casaccia et al (1989) conducted similar tests to Diringer, but with lesser concentration of the blood and covering the whole incubation period. Blood from donor hamsters with hamster-adapted 263K scrapie from intraperitoneal inoculation was concentrated so that each 50 µl of inoculum contained 0.2 ml blood equivalent. This was inoculated intracerebrally into groups of recipient hamsters. The level of infectivity was estimated from the mean incubation period of the hamsters that died of scrapie. Table II.3.5 gives the results scaled from the plot in Casaccia et al (1989). This indicates a relatively high level of infectivity in the first half of the incubation period, and a subsequent decline, with negligible infectivity at the end of the incubation period.

Table II.3.5 Infectivity in Blood by Casaccia et al

Time After Infection Of Donor (Assay) Animal (Days)	Recipient Animals Infected	Mean Incubation Period Of Recipient Animal (Days)	Estimated i/c log ID_{50}/0.05 ml
1	6/6	107	2.45
8	6/6	116	1.8
16	6/6	115	1.9
27	6/6	114	2.0
41	6/6	129	1.2
51	6/6	124	1.4
60	?/6	132	1.0
70	6/6	120	1.6
80	1/6	132	1.0
90	1/6	138	0.8
100	0/6	>160	-

II.3.4.5 Tests by Brown et al

Brown et al (1999) looked at blood infectivity during the pre-clinical and clinical phases of disease and conducted numerous bioassay experiments with blood from mice inoculated intracerebrally by a mouse-adapted strain of human TSE. The blood was then processed and inoculated intracerebrally in infectivity assays. Brown concluded that infectivity was present in the preclinical phase in the buffy coat, but was either absent or present in only trace amounts in the plasma or plasma fractions. Infectivity rose sharply at the onset of clinical signs in both the buffy coat and in the plasma. Table II.3.6 below illustrates that the experiments show that the preclinical infectivity is less than 8% of the clinical infectivity levels for buffy coat and less than 3% for plasma. If this is averaged out proportionate to

volumes, it gives a combined relative infectivity of preclinical to clinical infectivity for buffy coat/plasma of only 3%.

Table II.3.6 Comparison of Pre-clinical and Clinical Infectivity based on Brown et al 1999

	Average Preclinical Infectivity (IUs/ml)	Clinical Infectivity (IUs/ml)	% infectivity preclinical to clinical
Buffy Coat	8.2	106.0	7.7%
Plasma	0.6	21.9	2.7%
Cryo + I +II +III	0.7	-	-

II.3.4.6 Tests by Houston et al

Houston et al (2000) describes preliminary reports of one case of transmission of BSE to a sheep by transfusion of whole blood taken from a sheep asymptomatic from experimental BSE (this sheep was half way through the incubation period and was infected orally).

II.3.4.7 Interpretation

The available data suggests that the infectivity may remain stable, decrease or increase during the incubation period. However, the evidence for low infectivity at the beginning of the incubation period comes mainly from animals inoculated intracerebrally (Manuelidis, Kuroda and Brown). When considering vCJD infections from food or blood transfusions, the intraperitoneal inoculation used by Diringer and Casaccia may be more relevant. These both indicate a level of infectivity that is high at first, while Cassacia found that infectivity subsequently declines. Such a pattern might explain why several tests on blood from clinical cases have not detected infectivity. Also, the preliminary results from Houston indicate that there is sufficient infectivity in blood during the incubation period to enable infectivity to be transmitted by blood transfusion within the same species.

For the present study, it will be assumed that the level of infectivity remains constant through the incubation period. Since the levels estimated below are based on tests on blood from clinical cases, this may be an optimistic assumption. However, it is offset by assuming that infectivity levels in experimental CJD in mice are applicable to humans, which may be pessimistic.

II.3.5 The Level of Infectivity in Whole Blood

II.3.5.1 Tests by Brown et al (1998)

The most detailed available titration of infectivity in the blood is from tests by Brown et al (1998). Three sets of experiments were performed:

- A high input ("spiking") experiment, using hamster-adapted 263K scrapie. Infected hamster brain was added to human blood, which was then separated into components and inoculated intracerebrally at various dilutions into hamsters. Although an infectivity was estimated for whole blood in this experiment, the approach produces an artificially high

value which does not indicate the overall infectivity in normally infected blood. These tests can be used to indicate the relative level of infectivity in different blood components.

- A low input "endogenous" experiment, using mouse-adapted CJD. Blood from symptomatic mice inoculated intracerebrally was extracted, separated into components and inoculated intracerebrally at various dilutions into mice. Although no infections resulted from whole blood, these tests can be used to indicate the relative level of infectivity in most of the different blood components.
- Transfusion experiments, using hamster-adapted scrapie. Blood from symptomatic hamsters was inoculated intravenously and intracerebrally into other hamsters. Although the full results are not yet available, Rohwer (1997) has given some results. Rohwer has also reported in some presentations at conferences that he obtained one positive infection from blood inoculated intravenously. This was 1 out of 22 transmissions attempted. These results have not been published.

Available information on the test results is incomplete and unclear, and no single best-estimate of infectivity is available for whole blood. In the endogenous experiment, 45 ml of blood was obtained from mice with mouse-adapted CJD. Of this, 0.15% (i.e. 68 µl) was inoculated as whole blood into 11 mice, i.e. an average of 6 µl each. None of these 11 mice died. This suggests that the ID_{10} for whole blood is more than 6 µl. Assuming a linear dose-response function, this would indicate that the ID_{50} was more than 30µl, i.e. an infectivity less than 30 ID_{50}/ml. Interim results from Brown et al quoted a value of <76 ID/ml, but this does not appear in the final paper.

Rohwer (1997) stated that by adding the infectivity levels estimated in the individual blood components in the same tests "we get a titre for blood of about 10 infectious units (IU)/ml". Analysis of the infectivity of the plasma and buffy coat components from the low input experiment (Section II.3.6.2, Table II.3.11) indicates about 340 IU (680 ID_{50}) from a 45 ml blood sample (equivalent to 7.6 IU/ml), but the infectivity of the red cell component was not determined. Hence an infectivity for whole blood of 10 i/c IU/ml would be consistent with a 24% contribution from red cells.

For the transfusion experiments using hamster-adapted scrapie, Rohwer (1997) also stated "the titre in the blood by these experiments is about 2-10 infectious units/ml". This is assumed to refer to the intracerebral route, although this is not clear from the source. No complete results are available.

This source has the advantage that it included investigation of the infectivity in different blood components (see Section II.3.6.2). However, it has the disadvantage that reporting is incomplete and unclear, with no single infectivity result for whole blood. It only considers infectivity at the end of the incubation following intracerebral inoculation.

II.3.5.2 Tests by Brown et al (1999)

Brown et al (1999) conducted further experiments to assess the infectivity of buffy coat, plasma and plasma fractions, at both preclinical and symptomatic stages, as summarised in Table II.3.7 below, which compares infectivity with the levels determined in his previous experiment (II.3.5.1). Unfortunately no infectivity experiments would appear to have been

conducted on red blood cells or whole blood since 1998. However, infectivity of whole blood can be estimated from its components.

Table II.3.7 Comparison of Infectivity in Brown et al Experiments 1999 with 1998

	Brown et al 1999 Asymptomatic	Brown et al 1999 Symptomatic	Brown et al 1998 Symptomatic
Buffy Coat	16.4	212	88.8
Plasma	1.2	56.2	20.6

(All units in ID_{50}/ml component, where $2ID_{50}$/ml = 1 IU/ml

Both buffy coat and plasma contained higher infectivity levels during the symptomatic stage than those found in the earlier Brown et al (1998) experiments (by a factor of approximately 2.5). Brown et al considered that the higher infectivity levels in the buffy coat in the 1999 paper were expected, because in the earlier 1998 paper some mice receiving buffy coat had died with typical clinical signs but were not included in the infectivity calculations because their brains were not examined for PrP, hence death was not proven to have resulted from disease transmission. However, Brown (2001) considers that the higher symptomatic infectivity value found in the 1999 experiments for the buffy coat is "clearly an overestimate, or we would already have seen many cases of CJD in blood recipients". The experiments indicated that preclinical infectivity is less than 10% of the clinical infectivity levels.

II.3.5.3 Tests by Diringer

Diringer (1984) estimated the infectivity in the blood from donor hamsters with hamster-adapted 263K scrapie from intraperitoneal inoculation, early in the incubation period (Section II.3.4.3). Based on an incubation period range of 130-200 days, Diringer estimated an infectivity level of 5-50 i/c IU/ml.

This source has the advantage of considering infectivity during the incubation period following intraperitoneal inoculation. However, it has the disadvantage that it was based on scrapie, and that titration was not performed.

II.3.5.4 Tests by Casaccia et al

Casaccia et al (1989) conducted similar tests to Diringer, but with lesser concentration of the blood and covering the whole incubation period. Blood from donor hamsters with hamster-adapted 263K scrapie from intraperitoneal inoculation was concentrated so that each 50 µl of inoculum contained 0.2 ml blood equivalent. This was inoculated intracerebrally into groups of recipient hamsters. The level of infectivity was estimated from the mean incubation period of the hamsters that died of scrapie. Table II.3.3 gives the results.

The average infectivity during the first half of the incubation period was $10^{1.8}$ ID_{50}/0.05 ml inoculum (= 63 ID_{50}/0.05 ml inoculum). Since each inoculum contained 0.2 ml blood equivalent, this can be expressed as 310 ID_{50}/ml. The average over the whole incubation period would be approximately half of this.

This source has the advantage of considering infectivity during the whole incubation period following intraperitoneal inoculation. However, it has the disadvantage that it was based on scrapie, and that titration was not performed.

II.3.5.5 Previous Work on BSE

Previous work by DNV has used tests on various tissues from cattle with BSE (summarised by SEAC 1994 p65). These tests did not detect any infectivity in blood clots from cattle with BSE , or sheep or goats with scrapie. The detectability threshold of the tests gave an upper bound on the infectivity for all tests of $<10^1$ mouse i/c ID_{50} per ml. The previous DNV studies assumed that the infectivity of blood was 10% of the detectability threshold, i.e. 1 mouse i/c ID_{50} per ml bovine blood.

In order to estimate an infectivity within a single species, it is assumed that the species barrier between cattle and mice amounts to a reduction in efficiency of infection of 1000-fold, as in previous judgements on this issue. This value is highly uncertain. It indicates an infectivity within one species of 1000 bovine i/c ID_{50} per ml bovine blood.

This value is high compared to the other estimates. As it is not based on actual measurements of infectivity and includes a species barrier correction, it is considered much less reliable. However, it does show that the values from the other sources are consistent with (i.e. lower than) the values assumed in the previous work.

II.3.5.6 Tests by Bruce et al

Bruce et al (2001) conducted bioassay experiments on mice with plasma and buffy coat from vCJD patients. The experiments failed to detect infectivity in either source but could be used as an upper limit for infectivity, as follows. For plasma, each animal received an undiluted 20 Linoculum. The absence of infections indicates that 0.02 mls of plasma must contain much less than 1 ID_{50}. In total 39 animals were inoculated with plasma from 4 vCJD patients. This indicates a probability of infection less than 1/39 = 0.026. Assuming a linear dose-response model (Section II.6), the limit of detection for the ID_{50} (i.e. the dose with 0.5 probability of infection) could be estimated as 0.02 x 0.5 / 0.026 = 0.38 ml (ie. it is estimated that 0.38 mls contains 1 ID_{50}). Hence the limit of detection is estimated as 1/0.38 = 2.6 mouse ic ID_{50}/ml. Since the buffy coat was diluted two-fold before inoculation into a similar number of animals, the limit of detection for it would be approximately a factor of 2 higher.

These experiments were conducted across the human-mouse species barrier. Bruce et al assume that this barrier is approximately 500, based on the cattle-mouse species barrier. Following this assumption, the limit of detection for infectivity within a species would be 2.6 x 500 = 1,320 human ic ID_{50}/ml for plasma. This is significantly more than the infectivity assumed in the present report for plasma and buffy coat, hence it is not surprising that the experiment did not detect any infectivity, and the upper limit is of very limited use.

II.3.5.7 Wadsworth et al

Wadsworth et al (2001) indicates that although highly sensitive immuno-blotting analysis methods for detection of PrP have improved, they are not yet sensitive enough to be used for

estimation of blood infectivity unless spiking has taken place to raise infectivity levels. Additionally, the quantitative relationship between PrP and infectivity is uncertain.

II.3.5.8 Selection of Estimate

The above approaches are compared in Table II.3.8. In order to put them into comparable units, the values are converted using 1 IU = 2 ID_{50}. They give estimates of infectivity in blood ranging from 4 to 300 i/c ID_{50}/ml. The experiments by Brown et al (1998 and 1999) are at the lower end of this range.

Since these experiments all used species and disease strains that have been selected to achieve transmission, it is likely that they would provide an over-estimate of the infectivity of vCJD in humans. Additionally, infectivity in brains from patients dying of CJD averages about 40,000 IU/g (=80,000 ID_{50}/g) (Brown et al 1999) whereas infectivity levels were 25 to 250 times greater in the two mice brain pools used in Brown et al (1999) experiments and the higher of the brain pools was associated with the higher blood infectivity levels. Therefore, a value of 10 i/c ID_{50}/ml is used for this study, being at the lower end of the range. The full range of 4 to 300 i/c ID_{50}/ml is used for sensitivity tests.

Table II.3.8 Estimates of Overall Infectivity in Blood

Source	Original Estimate	Estimate In Comparable Units
Rohwer (1997) hamster adapted scrapie	2-10 hamster i/c IU/ml mouse blood	4-20 hamster i/c ID_{50}/ml hamster blood
Brown et al (1998) mouse adapted CJD ("Endogenous experiment")	10-15 mouse i/c IU/ml mouse blood	20-30 mouse i/c ID_{50}/ml mouse blood
Brown et al (1999) mouse adapted CJD (range allows for pre-clinical and clinical infectivity)	0.5-15 mouse i/c IU/ml mouse blood (buffy coat/plasma only)	1-30 mouse i/c ID_{50}/ml mouse blood (buffy coat /plasma only)
Casaccia et al (1989) hamster scrapie tests (first half of incubation period)	1.8 log hamster ID_{50}/0.2 ml hamster blood equivalent	310 hamster i/c ID_{50}/ml hamster blood
Diringer (1984) hamster scrapie tests (first third of incubation period)	5-50 hamster i/c IU/ml hamster blood equivalent	10-100 hamster i/c ID_{50}/ml hamster blood

II.3.5.9 Conversion to Intravenous Route

All the above estimates are based on intracerebral (i/c) inoculation, which is in general the most efficient route for transmitting infection. Intravenous (i/v) inoculation is reported to be 10 times less efficient than the i/c route, based on other tests with mouse-adapted scrapie (Kimberlin 1996). Brown et al (1999) conducted a comparison between i/v and i/c administration routes of inoculation and found a 7-fold reduction in infectivity by the i/v route using plasma from a murine model and a 5 fold reduction using buffy coat from a murine model. The research was conducted with blood from mice that had earlier been infected with a mouse-adapted strain of human TSE.

DNV propose a reduction factor of 5 for conversion from i/c to i/v inoculation. This will be used for whole blood, blood components and plasma fractions.

Combined with the estimate of i/c infectivity of 10 i/c ID_{50}/ml blood above, this gives 2 i/v ID_{50}/ml blood, with a range of approximately 0.2 to 60 i/v ID_{50} /ml.

Brown et al (1998) suggested that the i/v route might be 100 times less efficient, based on earlier work by Kimberlin and this is used as a sensitivity test to indicate the uncertainty in this parameter.

II.3.6 The Level of Infectivity in Blood Components

II.3.6.1 Based on White Cell Content

The recent work implicating B lymphocytes in the development of experimental scrapie suggests that infectivity in vCJD may be contained in these cells. On the other hand, other experiments using different strains of scrapie are showing no involvement of B cells in transport of infectivity. It may be, therefore, that different strains of infectious agents target different cells of the lympho-reticular system, as they do in the brain.

At present it is not clear whether the vCJD strain is carried in the B cells of humans. Nevertheless, one possibility is that, when blood is segregated into components and processed into blood products, infectivity may remain in the products in proportion to the number of B cells present. There is no data on the numbers of B cells in each blood product, so these are assumed to be in proportion to the number of white cells.

The mean white cell content in a 450 ml donation of whole blood is taken as 4×10^9 cells per unit (Appendix I.4.2), i.e. 8.9×10^9 cells per litre. If the infectivity is taken as 2 i/v ID_{50}/ml and assumed proportional to the number of white cells, it can be expressed as 2.2×10^{-7} ID_{50} per white cell. The infectivity in each blood component can then be estimated from the white cell content (Appendix I.4) as summarised in Table II.3.9.

Table II.3.9 Infectivity Based on White Cell Content

Blood Product	Volume (ml/unit)	Fraction Of Volume	WBCs (per unit)	Infectivity (ID50/unit)	Infectivity Concentration (ID50/ml)
Whole blood	450	1.000	$4x\ 10^9$	900	2
Plasma	225	0.500	$4x10^6$	0.9	0.004
Plasma (filtered)	225	0.500	1,000	0.0002	$1x10^{-6}$
Red cells (with buffy coat)	225	0.500	$4x10^9$	900	4
Red cells (buffy coat removed)	212	0.470	$5x10^8$	110	0.5
Red cells (leucodepleted)	212	0.470	$5x10^5$	0.11	0.0005
Buffy coat	14	0.030	$3.5x10^9$	800	5.8
Platelets (unfiltered)*	0.75	0.002	$6x10^6$	1.4	1.9
Platelets (filtered)*	0.75	0.002	1×10^6	0.3	0.4

* Figures are for platelets as derived from 1 unit of blood, while therapeutic platelet doses are typically derived from the pooling of 4 individual donations. Figures do not include the plasma present within a platelet dose. Also, it should be noted that a filtered platelets therapeutic dose has a UK specification that 100% of units have a WBC content of less than 5×10^6 per therapeutic dose (1.2×10^6 per unit of blood); routine testing indicates an average WBC count of 5×10^5. Unfiltered platelets refer to the removal of buffy coat.

This approach may under-estimate the level of infectivity in plasma and in leucodepleted red cells because it is possible that infectivity could be present in other cellular components, not

just white cells, and also in fragments of B cells that result from the decay of white cells. Protein is also a component of plasma, so it is also possible that prion exists in soluble form, although previous studies have assumed that prion only exists as a solid particle. Hence this approach may be regarded as an extreme estimate, optimistic for plasma and leucodepleted components, and pessimistic for red cells with buffy coat attached.

II.3.6.2 Tests by Brown et al (1998)

Brown et al (1998) investigated the distribution of infectivity in different blood components. Two separate experimental methods were used - a high input ('spiking') experiment and a low input endogenous one.

In their high input ("spiking") experiment, artificially high levels of hamster-adapted scrapie infectivity from brain homogenate were added to normal human blood, which was separated by centrifugation into red cells, white cells/platelets and plasma components, and the plasma was subjected to Cohn fractionation, as used by the American Red Cross. Titrations in each component were then determined as shown in Table II.3.10.

These results show that the majority of the infectivity went into the red cell component, although the infectivity concentration was higher in the white cell/platelet component. Only 32% of the infectivity in whole blood was recovered in the blood components, and only 1.5% of the infectivity in plasma was recovered in the plasma fractions. While the latter effect could be a genuine effect of fractionation, a more cautious interpretation might be that this method under-estimates the infectivity in the components. As a sensitivity test, the percentages could be adjusted in proportion so as to sum to 100%.

This experiment is questionable because the partitioning of the infectivity may reflect the properties of the brain homogenate, which would be very different from blood. However, the results of the experiment are broadly consistent with the more realistic endogenous experiment described below.

Table II.3.10 Infectivity Based on Spiked Blood Experiments

Component	Specimen Quantity	Infectivity Concentration (log ID_{50}/ml) or log ID_{50}/g)	Total Infectivity (log ID_{50})	% Of Infectivity In Whole Blood
Whole blood	46.8 ml	8.3	9.3×10^9	100 %
Red cells	20.0 ml	8.0	2.0×10^9	22 %
White cells/platelets	2.0 ml	8.5	6.3×10^8	7 %
Plasma	24.0 ml	7.1	3.0×10^8	3 %
Cryoprecipitate	0.26 g*	6.6	1.0×10^6	0.71 % #
Fraction I+II+III	0.93 g*	6.1	1.2×10^6	0.86 % #
Fraction $IV_1 + IV_4$	0.87 g*	4.0	8.7×10^3	0.006 % #
Fraction V	1.66 g*	2.5	0.5×10^3	0.0004 % #

* Based on fractionation of an 11 ml plasma sample.
# Percentage of infectivity in plasma.

In the endogenous experiment, blood from mice with mouse-adapted CJD was separated into components and fractionated as above. Titrations were not carried out, but the infectivity in

the blood components can be estimated from results provided by Rohwer as shown in Table II.3.11.

Table II.3.11 Infectivity Based on Low Input Experiments

Component	Vol (ml)	Positive/ Inoculated Animals	Total Infectivity (ID_{50})**	% Of Total Infectivity	Infectivity Concentration (ID_{50}/ml or ID_{50}/g)
Whole blood	45	0/11	-	-	-
Red cells	18	0/7	-	-	-
White cells/platelets	3.5	2/12	200.6	29.4	57.4
Plasma pellet*	0.2	4/23	14.6	2	73
Plasma	22.6	8/124	466	68.4	20.6
Cryoprecipitate	0.15	5/11	46	9.6 #	308
Fraction I+II+III	0.4	6/43	36	7.4 #	90
Fraction IV_1 + IV_4	0.86	0/86	-	-	
Fraction V	1.22	0/94	-	-	-

* White cell pellet recovered by centrifugation of plasma.

* * Based on Poisson Titre approach

Percentage of infectivity in plasma + white cell pellet

These results show that a large proportion of infectivity was in the pellet extracted from the plasma component by centrifugation. It is assumed that this infectivity would normally remain in the plasma component. The small number of animals inoculated with red cells mean that no infectivity can be determined for this component, but the results are consistent with a high level of infectivity in the red cells, as in the spiking experiment.

Only 17% of the infectivity in plasma (including the plasma pellet) was recovered in the plasma fractions. As a sensitivity test, the percentages could be adjusted in proportion so as to sum to 100%, as for the spiking experiment.

The results show a low level of infectivity in Fractions IV and V, but do not quantify it. However, they are consistent with the results from the spiking experiment, and could be combined with it to quantify the infectivity in these fractions.

II.3.6.3 Tests by Brown et al (1999)

Brown et al (1999) conducted further experiments to determine infectivity of buffy coat and plasma as summarised previously in Table II.3.7. Unfortunately, no experiments were conducted on red blood cells. The relative infectivity of the plasma and buffy coat during the symptomatic stage are very similar in both the 1998 and 1999 tests.

II.3.6.4 Tests by Taylor et al (2000)

Taylor et al (2000) developed a mouse-adapted BSE strain (301v) model which gave preliminary results of plasma infectivity of 5 ic ID_{50}/ml, which is consistent with the value of 10 i/c ID_{50}/ml for whole blood selected in Section II.3.5.8.

II.3.6.5 Other Experiments

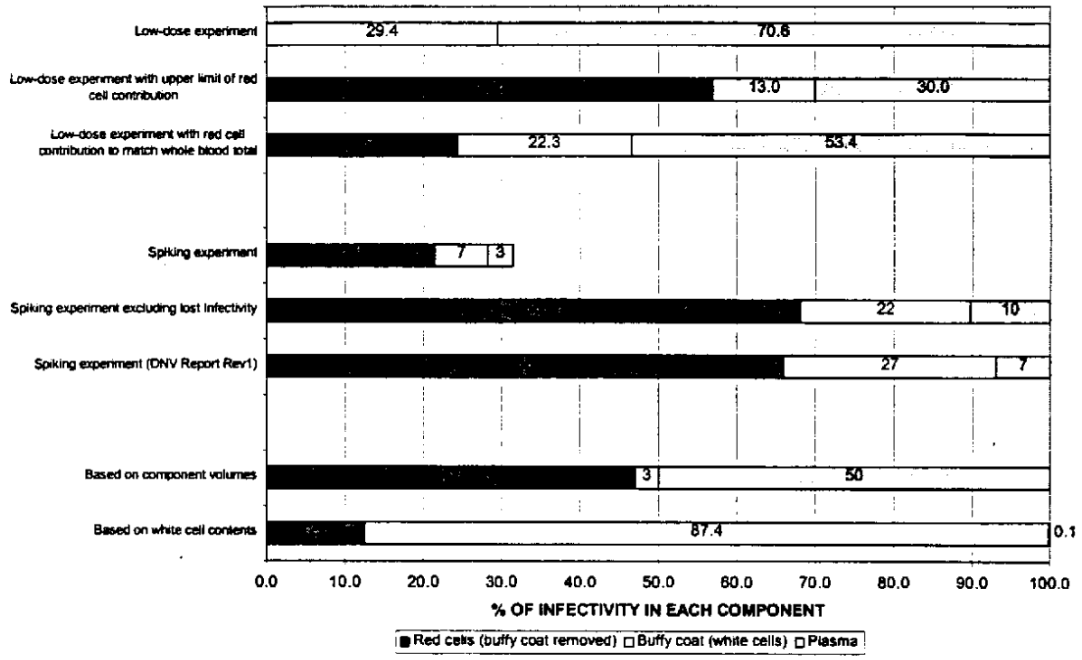
Several experiments that indicated infectivity in blood used buffy coat (platelets and white cells) (see Tables II.3.1 and II.3.2). The fact that they demonstrated infectivity in the buffy coat does not prove that there is none in the other components.

Other tests indicated infectivity in plasma from a sporadic CJD case (Tamai et al 1992) and serum (i.e. plasma with fibrinogen removed) from experimental scrapie in sheep and rats (reviewed by Brown 1995). It is possible that white cells were incompletely removed from these components, so this does not prove that infectivity is present outside white cells.

II.3.6.6 Comparison of Estimates

Figure II.3.1 compares various ways of estimating the breakdown of infectivity in whole blood donations between the different components.

Figure II.3.1 Comparison of Estimates of Infectivity in Blood Components



The methods are:

1. Direct analysis of low input experiment (Brown et al 1998) with zero for red cell component. Note that Brown et al experiments in 1999 gave similar proportions for infectivity breakdown , hence they are not shown on Figure II.3.1.

2. Analysis of low input experiment but with 1 out of 7 inoculated animals assumed infected, giving a pessimistic estimate of the red cell contribution.

3. Analysis of low input experiment (Brown et al 1998) with a red cell contribution of 24%, sufficient to give an overall infectivity of 10 IU/ml (see Section II.3.5.1). This is considered to be the best approach, and the infectivity proportions are then combined with the whole blood infectivity of 2 i/v ID_{50}/ml (as derived in Section II.3.5.9) to give infectivity values for each blood component.

4. Direct analysis of spiking experiment with no correction for lost infectivity.

5. Analysis of spiking experiment with percentages increased to eliminate lost infectivity. This is used as a sensitivity test, as it has the largest red cell component.

6. Analysis of previous draft paper on spiking experiment with percentages increased to eliminate lost infectivity, as used in Revision 1 of this report.

7. Infectivity concentrations assumed to be the same for all components, giving percentages proportional to the component volumes.

8. Infectivity in each component assumed proportional to white cell content (Section II.3.6.1).

The wide variation between the possible approaches illustrates the substantial uncertainties involved.

Using the Brown et al data leads to the conclusion that, although the highest infectivity *concentration* is in the buffy coat, much of the *total* infectivity remains in the red cell and plasma components. Clearly it would be desirable to have better quality data on these aspects.

The selected infectivity breakdown based on Approach 3 above is summarised in Table II.3.12.

Table II.3.12 Selected Infectivity of Blood Components Based on Brown et al (1998)

Blood Product	Volume (ml/unit)	Infectivity (ID50/unit)*	Infectivity Concentration (ID50/ml)*
Whole blood	450	900	2.0
Plasma	225	480 (=53.4% of 900)**	2.1
Red cells	212	219 (=24.3% of 900)**	1.0
Buffy coat	14	201 (=22.3% of 900)**	14.9
Plasma (filtered)	225	5 ***	0.02
Red cells (leucodepleted)	212	2***	0.01
Platelets (unfiltered) (4 donations)	3	20***	7
Platelets (filtered) (4 donations)	3	2***	0.7

* Note that infectivities are based on Intravenous

** See Section II 3.6.6, Approach 3

*** The basis for the filtered components is described in Section II.4.2.

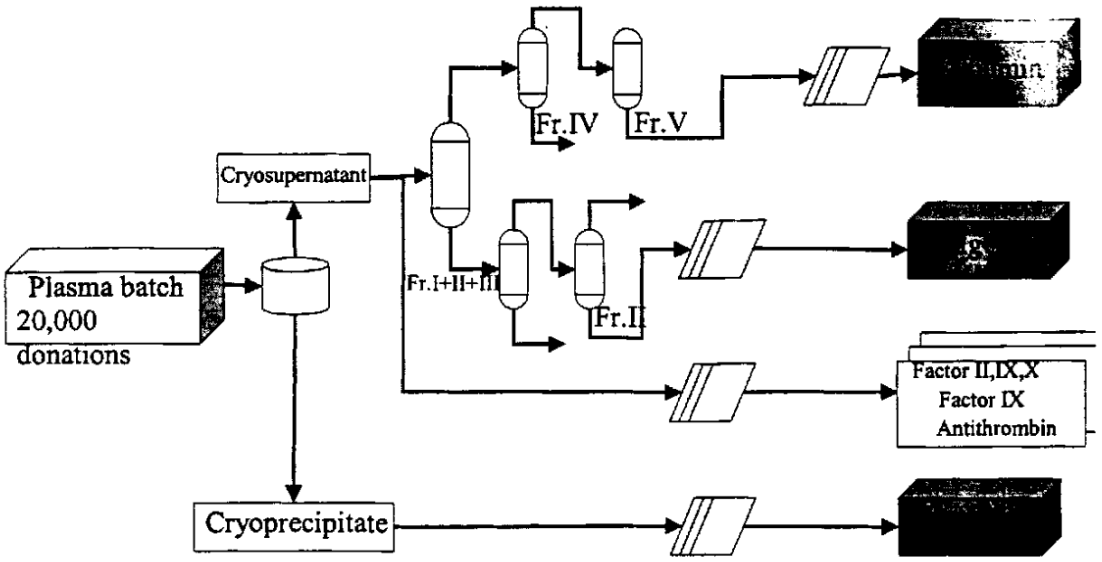
**** Figures for Platelets are for a therapeutic dose, which originates from the pooling of 4 separate donations (infectivities shown assume only 1 of the donations was from a patient with vCJD). Platelet figures do not include

data for the plasma present in a therapeutic platelet dose (typical platelet therapeutic dose is 300ml, comprising 297 ml plasma and 3 ml platelets)

II.3.7 The Level of Infectivity in Plasma Fractions

Figure II.3.2 provides an outline of the plasma fractionation process, illustrating the intermediate fractions that are used in manufacturing the final plasma derivatives.

Figure II.3.2 Outline of Plasma Fractionation



The low dose experiments by Brown et al (1998) estimate the infectivity in plasma fractions as detailed previously in Table II.3.11. Further experiments carried out by Brown et al (1999) estimated infectivity of the various fractions during both the pre-clinical and symptomatic stages of disease. It was concluded that plasma and its fractions contained only trace amounts during the pre-clinical stage (at the limit of assay recognition, and were detectable only by the inoculation of larger numbers of animals than usual). Cryoprecipitate levels at the symptomatic stage of disease contained low infectivity levels (but approximately twice the infectivity levels identified in the 1998 experiments). Also, the experiments detected trace infectivity levels in fractions IV and V at the symptomatic stage, unlike the 1998 endogenous experiment. Table II.3.13 summarises the experimental data and compares it with data from Brown's 1998 experiments.

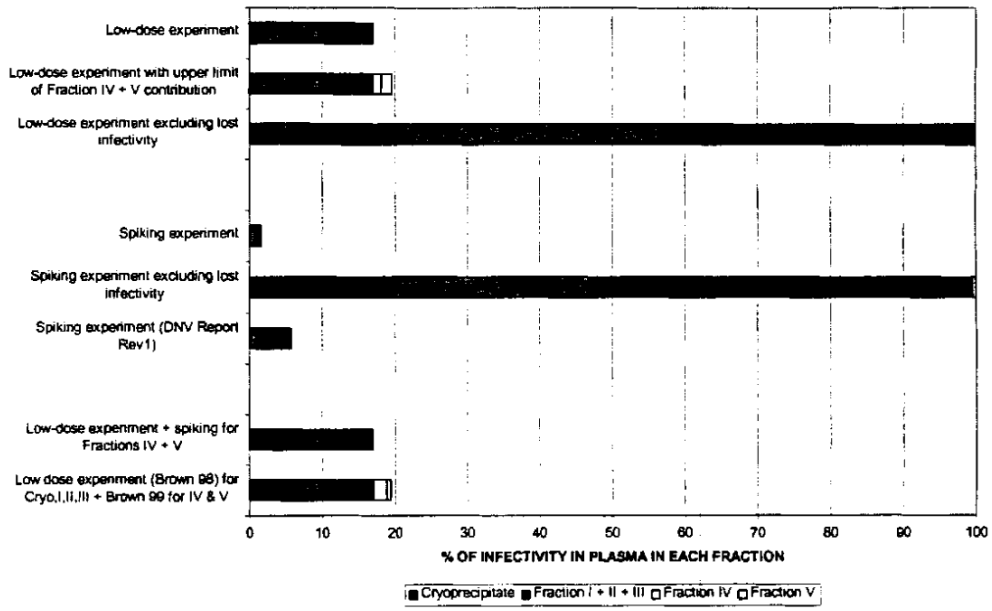
Table II.3.13 Comparison of Plasma Derivatives' Infectivity in Brown et al Experiments in 1999 with 1998

	Brown et al 1999 Asymptomatic	Brown et al 1999 Symptomatic	Brown et al 1998 Symptomatic
Plasma	1.2	56.2	20.6
Cryoprecipitate	1.4 (combined with I,II,III)	5.2	2.4
Fraction I+II+III *	1.4 (combined with cryo)	NT	1.6
IV	NT	1.0	< 0.22
V	NT	0.3	< 0.30
V supernatant	NT	ND	NT

Note that some infectivity levels for the 1999 experiments have been combined from the 2 pools of experiments to enable simple comparison of relative infectivities to be made. All units in ID_{50}/ml plasma.
NT = not tested
ND = not detected
* The Brown et al (1999) experiment also separated Fraction II from Fractions I and III, and most of the infectivity (95.6%) was found to remain with Fractions I and III.

There are several possible ways of analysing the available data, as shown in Figure II.3.3.

Figure II.3.3 Comparison of Estimates of Infectivity in Plasma Fractions



The methods are:

1. Direct analysis of low input experiment (Brown 1998) with zero for Fractions IV and V.

2. Analysis of low input experiment (Brown 1998) but with 1 of the inoculated animals for Fractions IV and V assumed infected, giving a pessimistic estimate of their contributions.

3. Analysis of low input experiment (Brown 1998) with percentages increased to eliminate lost infectivity.

4. Direct analysis of spiking experiment (Brown 1998) with no correction for lost infectivity.

5. Analysis of spiking experiment (Brown 1998) with percentages increased to eliminate lost infectivity.

6. Analysis of previous draft paper on spiking experiment, as used in Revision 1 of this report.

7. Analysis of low input experiment (Brown 1998) with contributions for Fraction IV (0.006% of plasma infectivity – see Table II.3.10) and Fraction V (0.0004%) from the spiking experiment.

8. Analysis of low input experiment (Brown 1998) provides data for Cryoprecipitate and Factors I, II and III with contributions provided by the low dose Brown et al experiment (1999) for Fractions IV (from Table II.3.13, the infectivity of Fraction IV is 19.2% of Cryoprecipitate = 1.85% of plasma infectivity) and Fraction V (0.55% of plasma). The infectivity of Fraction II can also be estimated from Brown et al's experiment (1999) as 4.6% of the infectivity of Fractions I, II, III.

Approach number 8 is the favoured approach.

The wide variation between the possible approaches illustrates the substantial uncertainties involved, particularly regarding the reduction in infectivity due to fractionation processes. However, all approaches indicate that most infectivity remaining after fractionation is in the cryoprecipitate and Fractions I and III, and that infectivity in Fractions II and V is relatively low.

The selected infectivity breakdown based on Approach 8 above is summarised in Table II.3.14.

Table II.3.14 Selected Infectivity in Plasma Fractions Based on Brown et al (1998 &1999)

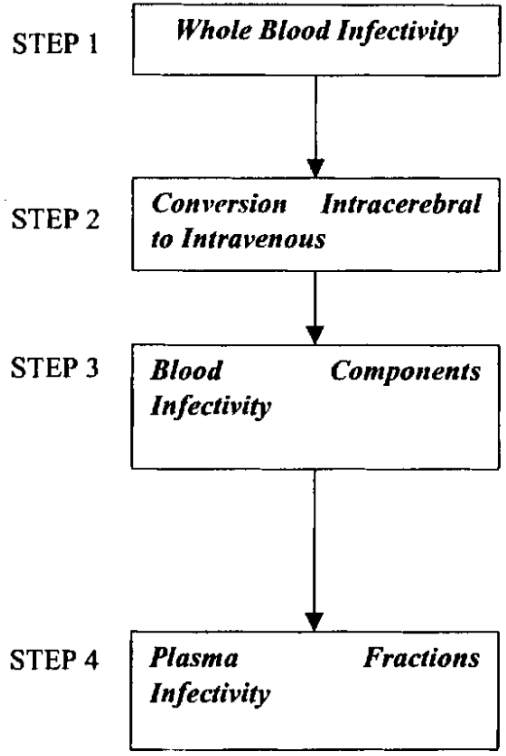
Plasma Fraction	Volume (ml/450 ml whole blood)	Infectivity (ID_{50}/unit whole blood)	Infectivity Concentration (ID_{50}/ml fraction)	Infectivity Concentration (ID50/g protein)**
Plasma	230	480	2.1	38.2
Cryoprecipitate	3	46	15.2	101
Fraction I + II + III	8	36	4.5	14.6
Fraction II	3.8	1.6	0.43	1.4
Fraction IV	17.5	8.9	0.5	1.7
Fraction V	24.8	2.6	0.1	0.34
Cryosupernatant	223	48	0.21	3.9

** This has been calculated from commercially sensitive information on the protein content in each relevant fraction (as supplied by BPL) and consequently full details are not provided here.

II.3.8 Summary of Derivation of Infectivity Levels

Figure II.3.4 overleaf summarises the key steps involved in the derivation of infectivity levels.

Figure II.3.4 Summary of Derivation of Infectivity Levels



	Summary	Relevant Section	Selection of Infectivity
STEP 1	Different experiments gave a range of infectivity from 4-300 ic ID_{50}/ml. Lower end of range selected (see Section II.3.5.8).	Section II.3.5.1 to II.3.5.8	10 i/c ID_{50}/ml whole blood
STEP 2	Five fold reduction factor chosen for conversion from i/c to i/v inoculation, based on experiments.	Section II.3.5.9	2 i/v ID_{50}/ml whole blood (900 i/v ID_{50}/unit whole blood)
STEP 3	A 24% infection contribution from RBC is derived as detailed in section II.3.5.1. For plasma and buffy coat, the Brown et al experiments give their proportional infectivity and combining this with 24% RBC, these are then related to the 900 iv ID_{50}/ unit whole blood infectivity derived in Step 2.	Section II.3.5.1 Section II.3.6.2 to II.3.6.6	Whole Blood: 900 i/v ID_{50}/unit Plasma: 480 i/v ID_{50}/unit whole blood RBC: 219 i/v ID_{50}/unit whole blood Buffy: 201 i/v ID_{50}/unit whole blood
STEP 4	Brown's 1998 and 1999 experimental data is used to determine the proportions of the plasma fractions infectivity and this is then related to the plasma infectivity derived in Step 3.	Section II.3.7	Cryoprecip: 46 i/v ID_{50}/unit whole blood Fract 1&III: 34.4 i/v ID_{50}/unit whole blood Fraction II: 1.6 i/v ID_{50}/unit whole blood Fraction IV: 8 i/v ID_{50}/unit whole blood FractionV: 2.6 i/v ID_{50}/unit whole blood Cryosuper: 48 i/v ID_{50}/unit whole blood

II.4 Effect of Blood Processing on Infectivity

II.4.1 Production of Blood Components

Primary processing of donated whole blood involves segregation into the main components (red cells, plasma and sometimes platelets) by centrifugation. The partitioning of infectivity can be estimated using the data from Section II.3.6. There is no evidence that any reduction in infectivity occurs in this processing except due to leucodepletion (see below). It is possible that natural breakdown of white cells following donation leads to progressive increase of infectivity in platelets, hence reducing the infectivity in red blood including buffy coat. However, any such effect would be within the range of values obtained by the different estimates in Figure II.3.1.

II.4.2 Leucodepletion

II.4.2.1 Leucodepletion of Red Cells

Leucodepletion involves removal of white cells from the blood by simple filtering. This is at present applied to some red cell units. It is believed to achieve a 4 log reduction in white cells compared to whole blood or red cell concentrate (Appendix I.4.5.5).

Since there is evidence that infectivity is related to white cells (Section II.3.6.1), it is possible that infectivity is proportional to white cell content. Hence a simple estimate of the effect of leucodepletion would be that it also achieved a 4 log reduction in infectivity compared to whole blood or red cell concentrate, as shown in Table II.3.9.

However, there are several reasons why infectivity may not reduce in proportion to white cell content:

- It is possible that some fragments of white cell membranes may pass through the filter. This is particularly likely if filtering is done at a late stage (immediately prior to transfusion rather than soon after donation), since white cells progressively break down during this time. However, NBS plan to filter within 48 hours of donation, which should greatly reduce the occurrence of membrane fragments. Preliminary results indicate that filtration is very effective.
- It is possible in theory that filtration could release attached prion from the surface of the white cells into the leucodepleted component, although there is no evidence that this occurs.
- The experiments by Brown et al (1998) show some infectivity in the buffy coat, which could be removed without leucodepletion. However, the filters used for leucodepletion would remove the majority of platelets.
- The experiments by Brown et al (1998 and 1999) also show considerable infectivity in the plasma, which is not consistent with the hypothesis that infectivity is proportional to white cell content.

In the absence of any experiments to show the effect of leucodepletion on infectivity, it is appropriate to assume that leucodepletion reduces the infectivity substantially, but by less than the reduction in white cell content. The infectivity in leucodepleted red cells is assumed to be 2 logs lower than red cells with buffy coat removed. Combining with the selected infectivity breakdown by blood component from Section II.3.6.5, this gives a 2.6 log reduction compared to whole blood. This is shown in Table II.3.12, and used in the present study as the base case.

II.4.2.2 Filtration of Plasma

Filtration of white cells from FFP units is not generally used at present in the NBS, but could achieve a 3 to 4 log reduction in white cells compared to unfiltered FFP units (Appendix I.4.7). Based on the similar arguments to those for leucodepletion of red cells above, the reduction in infectivity is assumed to be 2 logs (however, Brown et al (2001) suggests that WBC reduction does not consistently reduce the infectivity of already separated plasma).

II.4.2.3 Filtration of Platelets

After 3-component segregation, most of the white cells from a blood donation remain in the buffy coat. In order to meet the specification for filtrated platelet units (Appendix I.4.6.2), some degree of filtration is required, amounting to approximately 3 log reduction of white cells. Based on the similar arguments to those for leucodepletion of red cells above, the reduction in infectivity is assumed to be 2 logs.

For unfiltered platelet units, reduction in WBC content is of the order of 2 logs; the associated reduction in infectivity is assumed to be 1 log.

II.4.3 Production of Plasma Derivatives

II.4.3.1 General Approach

Production of the various plasma derivatives (see Figure II.3.2) involves various stages of fractionation, precipitation, centrifugation, filtration, virus inactivation, formulation and heat treatment. Several of these steps are intended to achieve a major reduction in viruses, and it is possible that they also have a significant effect on CJD infectivity. A number of studies to assess the removal of the TSE agent in plasma fractionation processes have been carried out by "spiking" the starting material with extracts from the brains of animals with a TSE (Foster et al 1998, 2000). These studies suggests that a significant reduction in infectivity is achieved. However, concern has been expressed that the characteristics of any infectivity that may be present in plasma may not be the same as those in the spiked material.

Three possible approaches for estimating the infectivity in plasma derivatives have been considered. The third approach is a worst case scenario.

Note: The 3 approaches assess infectivity in BPL products only, as they are applied to BPL product data (protein content), product yields, product dose size, plasma batch data etc. Infectivity of specific SNBTS products will require assessment of SNBTS data once the agreed approach has been defined.

Approach 1: Protein content. Infectivity concentrations based on values in the Brown et al experiments on endogenous infectivity in the blood of animal models (Table II.3.14) for the cryoprecipitate or other appropriate fraction, combined with the *protein contents* in the plasma derivatives. This in effect assumes that infectivity partitions between the finished product and the waste material in proportion to the protein contents of the two, which is believed to be a reasonable approach, neither pessimistic nor optimistic. It assumes no further reduction in infectivity from further filtration steps after the fractionation (apart from that resulting from the reduction in the protein content).

Approach 2: Largest single clearance factor. Infectivity based on the value for plasma in the Brown et al experiments (Table II.3.12), combined with an estimate of the TSE reduction capability of the process steps, so called TSE *clearance factors (CF)*. An estimate of the potential for plasma fractionation processes to remove TSE infectivity has been made by Foster et al (1998, 1999 and 2000) based on SNBTS production methods, and the results of studies on the behaviour of brain-derived infectivity. Foster estimated both CF cumulatively and also individual CF for single process steps. Further work by Foster et al (as yet unpublished) has since indicated that clearance is unlikely to be cumulative and hence *this document considers the highest single clearance step only.* In this approach, the reduction in product volume is included in the CF, which refers to total infectivity, not infectivity concentration. Table II.4.1 summarises the highest single CF as estimated by Foster's experiments for the SNBTS processing methods. It should be noted that the CF are derived from experiments based on SNBTS processes, but that the CF are being applied to BPL processes. Table II.4.1 includes estimates of the highest single CF that *may* result from BPL's processing techniques, as in some cases there are differences between the two processes. Where this is the case the reasoning behind the choice of highest single CF is given below Table II.4.1.

Approach 3: No additional clearance. Infectivity concentrations based on values in the Brown et al experiments (Table II.3.14) for the cryoprecipitate or other appropriate fraction, and it is assumed that there is *no further reduction* in infectivity. Hence all the load in infectivity present in the fraction is assumed to be present also in the appropriate derivative. This is a worst case, and unlikely, scenario. Further pessimism is introduced by assuming that, where a specific fraction is used to produce more than one derivative, it is possible that all the infectivity present in the fraction can potentially end up in any one of the derivatives.

Table II.4.1 Estimated Clearance Factors in Plasma Products (Foster 2000)

Product	SNBTS		BPL	
	Process Step resulting in Highest Single CF	CF (log_{10})	Process Step resulting in Highest Single CF	CF (log_{10})
Albumin	Fraction V depth filtration	4.5	Fraction V Depth filtration (1)	4
Immunoglobulins	Fraction I/III precipitation	4	Fraction I/III precipitation (2)	3
Factor IX Replenine	DEAE Sepharose chromatography	3	DEAE Sepharose chromatography	3
Factor II, IX , X (IXA)	DEAE Cellulose adsorption	3	DEAE cellulose adsorption	3
Thrombin(BPL Antithrombin)	Sepharose chromatography	3	DEAE cellulose adsorption (3)	3
Factor VIII (intermed) 8Y	-	-	Cryoprecipitation (4)	1
Factor VIII (high purity) Rep	SD+DEAE chromatography	3	Ion exchange chromatography (5)	4

CF have been adapted to allow for differences between BPL & SNBTS Processes as follows:

(1) Depth filtration at BPL (Cuno filter) is different to SNBTS (Seitz KS80) and although it is understood to be an effective filtration method, a more prudent 4 log CF has been assumed for BPL.

(2) BPL use a different Fraction I/III precipitation method (Kistler & Nichmann) to SNBTS (cold ethanol); to allow for BPL's method being less effective at removing infectivity, a reduced CF of 3 logs is selected.

(3) BPL produce anti-thrombin, not thrombin. However DEAE Cellulose adsorption is common to both processes and experiments have shown clearance of 3 logs.

(4) BPL's Factor VIII 8Y process is a simple process; however both cryoprecipitation and filtration are common to its production and SNBTS's Factor VIII Liberate process, and a minimum of 1 log clearance is common to both methods.

(5) BPL's high purity Factor VIII Replenate includes immunoaffinity chromatography and an ion exchange chromatography steps, which literature (Foster et al 2000) suggests may have a higher single CF (up to 6 logs) than SNBTS processing for Factor VIII Liberate. A more prudent increased CF of 4 logs is selected.

The application of these three approaches to the plasma derivatives is provided below. The calculations refer to derivatives made from batches of plasma pooled from 20,000 donations including one from a donor who went on to develop vCJD.

II.4.3.2 Factor II, IX,X (IXA)

Factor II,IX,X (IXA) is produced from cryosupernatant.

Using the *protein content approach,* cryosupernatant has an infectivity of 3.9 ID_{50}/g protein (from Table II.3.14) x the fraction of donations infected (1 in 20,000), which results in an infectivity of 1.9 x 10^{-4} ID_{50}/g protein. A typical 1250 iu dose contains approximately 0.42 g of Factor II,IX,X(IXA), hence the infectivity would be 8 x 10^{-5} ID_{50} per 1250 iu dose (or 6 x 10^{-8} ID_{50}/iu).

Using the highest single *clearance factor approach*, the infectivity in plasma is first estimated 481 ID_{50}/unit x the fraction of donations infected. If each batch of 20,000 donations included one from a donor who subsequently developed vCJD, the total infectivity resulting from 25 plasma batches (only 30% of batches produce Factor IX Replenine) would be 11,800 ID_{50} pa. Allowing for a thousand-fold reduction in infectivity as a result of processing (see Table II.4.1), the potential infectivity is therefore estimated to be 11.8 ID_{50}/pa in Factor IX

Replenine. Now 2,400 units of 1000 iu Factor IX Replenine are produced pa, therefore the infectivity is estimated as 5 x 10^{-3} ID_{50}/1250 iu dose (or 5 x 10^{-6} ID_{50}/iu).

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for cryosupernatant. Infectivity in Cryosupernatant is 47 ID_{50}/unit whole blood, and based on one contaminated donor per batch of 20,000 and 25 batches pa, infectivity in the Cryosupernatant equals 1,200 ID_{50} pa. There are 2,400 units of 1250 iu Factor IX Replenine produced pa, therefore infectivity for a typical dose of 1000 iu is 0.5 ID_{50} (or 4 x 10^{-4} ID_{50}/iu).

II.4.3.3 Factor IX (Replenine)

Factor IX Replenine is produced from cryosupernatant (see Appendix I.5.4).

Using the *protein content approach,* cryosupernatant has an infectivity of 3.9 ID_{50}/g protein (from Table II.3.14) x the fraction of donations infected (1 in 20,000), which results in an infectivity of 1.9 x 10^{-4} ID_{50}/g protein, or 1.6 x 10^{-6} ID_{50} per 1000 iu dose (1.6 x 10^{-9} ID_{50}/iu).

Using the highest single *clearance factor approach*, the infectivity in plasma is first estimated 481 ID_{50}/unit x the fraction of donations infected. If each batch of 20,000 donations included one from a donor who subsequently developed vCJD, the total infectivity resulting from 25 plasma batches (only 30% of batches produce Factor IX Replenine) would be 11,800 ID_{50} pa. Allowing for a thousand-fold reduction in infectivity as a result of processing (see Table II.4.1), the potential infectivity is therefore estimated to be 11.8 ID_{50}/pa in Factor IX Replenine. Now 16,000 units of 1000 iu Factor IX Replenine are produced pa, therefore the infectivity is estimated as 7 x 10^{-4} ID_{50}/1000 iu dose (or 7 x 10^{-7} ID_{50}/iu).

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for cryosupernatant. Infectivity in Cryosupernatant is 47 ID_{50}/unit whole blood, and based on one contaminated donor per batch of 20,000 and 25 batches pa, infectivity in the Cryosupernatant equals 1,200 ID_{50} pa. There are 16,000 units of 1000 iu Factor IX Replenine produced pa, therefore infectivity for a typical dose of 1000 iu is 0.07 ID_{50} (or 7 x 10^{-5} ID_{50}/iu).

II.4.3.4 Anti-thrombin

Anti-thrombin is produced from cryosupernatant.

Using the *protein content approach,* cryosupernatant has an infectivity of 3.9 ID_{50}/g protein (from Table II.3.14) x the fraction of donations infected (1 in 20,000), which results in an infectivity of 1.9 x 10^{-4} ID_{50}/g protein. A typical 7000iu dose contains approximately 2.33g of protein. Hence the infectivity would be 4.5 x 10^{-4} ID_{50} per 7000 iu dose (or 6.5 x 10^{-8} ID_{50}/iu).

Using the highest single *clearance factor approach*, the infectivity in plasma is first estimated 481 ID_{50}/unit x the fraction of donations infected. If each batch of 20,000 donations included one from a donor who subsequently developed vCJD, the total infectivity resulting from 8 plasma batches (only 10% of batches produce Anti-thrombin) would be 3,950 ID_{50} pa. Allowing for a thousand-fold reduction in infectivity as a result of processing, the potential infectivity is therefore estimated to be 3.9 ID_{50}/pa in Anti-thrombin. Now 71 units of 7000 iu

Anti-thrombin are produced pa, therefore the infectivity is estimated as 0.055 ID_{50}/7000 iu dose (or 7.8 x 10^{-6} ID_{50}/iu).

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for cryosupernatant. Infectivity in Cryosupernatant is 47 ID_{50}/unit whole blood, and based on one contaminated donor per batch of 20,000 and 8 batches pa, infectivity in the Cryosupernatant equals 385 ID_{50} pa. There are 71 units of 7000 iu Anti-thrombin produced pa, therefore infectivity for a typical dose of 7000 iu is 5.4 ID_{50} (or 7.8 x 10^{-4} ID_{50}/iu).

II.4.3.5 Factor VIII Y

Factor VIII Y is produced from cryoprecipitate (see Appendix I.5.3).

Using the *protein content approach*, the infectivity concentration in Factor VIIIY resulting from cryoprecipitate is estimated as 101 ID_{50}/g protein (from Table II.3.14) x the fraction of donations infected (1 in 20,000), which results in an infectivity of 5 x 10^{-3} ID_{50}/g protein. A typical 2000 iu dose contains approximately 0.48 g of protein. Hence the infectivity would be 2.4 x 10^{-3} ID_{50} per 2000 iu dose (or 1.2 x 10^{-6} ID_{50} per iu).

Using the highest single *clearance factor approach*, the infectivity in plasma is first estimated 481 ID_{50}/unit (from Table II.3.12) x the fraction of donations infected. If each batch of 20,000 donations included one from a donor who subsequently developed vCJD, the total infectivity resulting from 29 plasma batches (only 35% of batches produce Factor VIII Y) would be 13,800 ID_{50} pa. Allowing for a ten fold reduction in infectivity as a result of processing, the potential infectivity is therefore estimated to be 1,380 ID_{50} /pa in Factor VIII Y. Now 17,000 units of 2000 iu Factor VIII Y are produced pa, therefore the infectivity is estimated as 0.08 ID_{50}/2000 iu dose (or 4 x 10^{-5} ID_{50}/iu).

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for cryoprecipitate. Infectivity in Cryoprecipitate is 46 ID_{50}/unit whole blood, and based on one contaminated donor per batch of 20,000 and 29 batches pa, infectivity in the Cryoprecipitate equals 1,330 ID_{50} pa. There are 17,000 units of 2000 iu Factor VIII Y produced pa, therefore infectivity for a typical dose of 2000 iu is 0.08 ID_{50} (or 4 x 10^{-5} ID_{50}/iu).

II.4.3.6 Factor VIII- Replenate

Factor VIII Replanate is produced from both cryoprecipitate and albumin (see Appendix I.5.3).

Using the *protein content approach*, the infectivity concentration in Factor VIII Replenate results from both cryoprecipitate and albumin. Considering *cryoprecipitate* first, this is estimated as 101 ID_{50}/g protein (from Table II.3.14) x the fraction of donations infected (1 in 20,000), which results in an infectivity of 5 x $10^{-3}$$ID_{50}$/g protein. A typical 2000 iu dose contains approximately 0.8 mg of Factor VIII. Hence the infectivity would be 4 x 10^{-6} ID_{50} per 2000 iu dose (due to the cryoprecipitate). Next considering the infectivity resulting from the *albumin*, this originates from Fraction V, with an infectivity of 0.34/20,000 = 1.7 x 10^{-5}

ID_{50}/g protein. A typical 2000 iu dose contains approximately 400 mg protein, hence the infectivity resulting from the albumin would be 6.8 x 10^{-6} ID_{50}/dose. Adding the two sources of infectivity together gives an infectivity of 1.1 x 10^{-5} ID_{50}/ dose (or 5.5 x 10^{-9} ID_{50}/iu).

Using the highest single *clearance factor approach*, the infectivity in plasma is first estimated 481 ID_{50}/unit (from Table II.3.12) x the fraction of donations infected. If each batch of 20,000 donations included one from a donor who subsequently developed vCJD, the total infectivity resulting from 53 plasma batches (only 65% of batches produce Factor VII Replenate) would be 25,500 ID_{50} pa. Allowing for a ten thousand-fold reduction in infectivity as a result of processing, the potential infectivity is therefore estimated to be 2.5 ID_{50} /pa in Factor VIII Replenate. Now 31,000 units of 2000 iu Factor VIII Replenate are produced pa, therefore the infectivity is estimated as 8 x 10^{-5} ID_{50}/2000 iu dose (or 4 x 10^{-8} ID_{50}/iu). The extra contribution from albumin is negligible.

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for Cryoprecipitate, cryosupernatant, Fractions II and V. The infectivity concentration in Factor VIII Replenate results from both cryoprecipitate and albumin, but for simplicity sake, and because the bulk of the infectivity will result from the cryoprecipitate, only this is examined here. Infectivity in Cryoprecipitate is 46 ID_{50}/unit whole blood, and based on one contaminated donor per batch of 20,000 and 53 batches pa, infectivity in the Cryoprecipitate equals 2,400 ID_{50} pa. There are 31,000 units of 2000 iu Factor VIII Replenate produced pa, therefore infectivity for a typical dose of 2000 iu is 0.08 ID_{50} (or 4 x 10^{-5} ID_{50}/iu).

II.4.3.7 Albumin

Using the *protein content approach*, the infectivity concentration in albumin is taken as the same as in Fraction V paste, 0.34 ID_{50}/g protein (from Table II.3.14) x the fraction of donations infected (1 in 20,000), which equals 1.7 x 10^{-5} ID_{50}/g protein. A bottle of albumin contains approximately 20g of albumin. Hence the infectivity of a 20 g bottle would be 3.4 x 10^{-4} ID_{50}.

Using the highest single *clearance factor approach*, the infectivity in plasma is first estimated as 481 ID_{50}/unit (as above) if all donations were infected. If each batch of 20,000 donations included one from a donor who subsequently developed vCJD, the total infectivity resulting from 82 plasma batches would be 39,500 ID_{50} pa.The infectivity in albumin is taken as 4 logs less than in plasma (from Table II.4.1). Hence the corresponding infectivity in albumin would be 3.9 ID_{50}/year, which translates into an infectivity of 5.1 x 10^{-7} ID_{50}/g of albumin produced (7.6 x 10^{6} g of albumin produced pa). The infectivity in a single 100ml bottle containing 20g of albumin is therefore approximately 1 x 10^{-5} ID_{50} .

The pessimistic approach for Albumin assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for Fraction V. Fraction V has an infectivity of 2.6 ID_{50}/unit whole blood (Appendix II Table II.3.14) and based on one contaminated donor per batch and 82 batches pa, infectivity in Fraction V equals 220 ID_{50} pa. There are 7,600 kg of Albumin produced pa, therefore infectivity is 2.8 x 10^{-5} ID_{50}/g. Based on a typical dose of 500ml of 4.5% albumin, an infectivity of 6.5 x 10^{-4} ID_{50} is estimated.

II.4.3.8 Immunoglobulins (iv)

IgG (iv) is produced from both Factor II and also contains albumin (see Appendix I.5.6).

Using the *protein content approach*, the infectivity concentration in IgG (iv) results from both Factor II and albumin. Considering Factor II first, this is estimated as 1.4 ID_{50}/g protein (from Table II.3.14) x the fraction of donations infected (1 in 20,000), which results in an infectivity of 7 x 10^{-5} ID_{50}/g protein. A typical 90 g dose of IgG contains 90g of IgG (iv) derived from Factor II. Hence the infectivity would be 6.5 x 10^{-3} ID_{50} per 90g dose (due to Factor II). Next considering the infectivity resulting from the *albumin*, this originates from Fraction V, with an infectivity of 0.34/20,000 = 1.7 x 10^{-5} ID_{50}/g protein. A typical 90g dose also contains approximately 36g albumin derived protein, hence the infectivity resulting from the albumin would be 6 x 10^{-4} ID_{50}/dose. Adding the two sources of infectivity together gives an infectivity of 7.1 x 10^{-3} ID_{50}/ 90g dose.

Using the highest single *clearance factor approach*, the infectivity in plasma is first estimated 481 ID_{50}/unit (from Table II.3.12) x the fraction of donations infected. If each batch of 20,000 donations included one from a donor who subsequently developed vCJD, the total infectivity resulting from 41 plasma batches (only 50% of batches produce Factor VII Replenate) would be 19,700 ID_{50} pa. Allowing for a thousand-fold reduction in infectivity as a result of processing, the potential infectivity is therefore estimated to be 19.7 ID_{50} /pa in IgG (iv). Now 3,111 units of 90g IgG are produced pa, therefore the infectivity is estimated as 6.4 x 10^{-3} ID_{50}/90g dose (or 7 x 10^{-5} ID_{50}/g). The extra contribution from albumin is negligible.

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for Factors II and V. The infectivity concentration in IgG (iv) results from both Factor II and albumin, but for simplicity sake, and because the bulk of the infectivity will result from Factor II, only this is examined here. Infectivity in Factor II is 1.6 ID_{50}/unit whole blood, and based on one contaminated donor per batch of 20,000 and 41 batches pa, infectivity in Factor II equals 66 ID_{50} pa. There are 3,111 units of 90g IgG (iv) produced pa, therefore infectivity for a typical dose of 90g is 0.022 ID_{50} (or 2 x 10^{-4} ID_{50}/g).

II.4.3.9 Immunoglobulins (im)

IgG (im) is produced from Factor II.

Using the *protein content approach*, the infectivity concentration in IgG (im) from Factor II is estimated as 1.4 ID_{50}/g protein (from Table II.3.14) x the fraction of donations infected (1 in 20,000), which results in an infectivity of 7 x 10^{-5} ID_{50}/g protein. Hence the infectivity would be 1.8 x 10^{-5} ID_{50} per 250mg dose.

Using the highest single *clearance factor approach*, the infectivity in plasma is first estimated 481 ID_{50}/unit (from Table II.3.12) x the fraction of donations infected. If each batch of 20,000 donations included one from a donor who subsequently developed vCJD, the total infectivity resulting from 8 plasma batches (only 10% of batches produce IgG (im)) would be 3,850 ID_{50}

pa. Allowing for a thousand-fold reduction in infectivity as a result of processing, the potential infectivity is therefore estimated to be 3.9 ID_{50} /pa in IgG (iv). Now 110,000 units of 250mg IgG are produced pa, therefore the infectivity is estimated as 3.5×10^{-5} ID_{50}/250mg dose (or 1.4×10^{-4} ID_{50}/g).

The pessimistic approach assumes there is *no further reduction in infectivity* load beyond the data available from Brown et al's experiments for Factor II. Infectivity in Factor II is 1.6 ID_{50}/unit whole blood, and based on one contaminated donor per batch of 20,000 and 8 batches pa, infectivity in Factor II equals 13 ID_{50} pa. There are 110,000 units of 250mg IgG (iv) produced pa, therefore infectivity for a typical dose of 250mg is 1.2×10^{-4} ID_{50} (or 5×10^{-4} ID_{50}/g).

II.4.3.10 Estimate of Infectivity in Plasma Derivatives

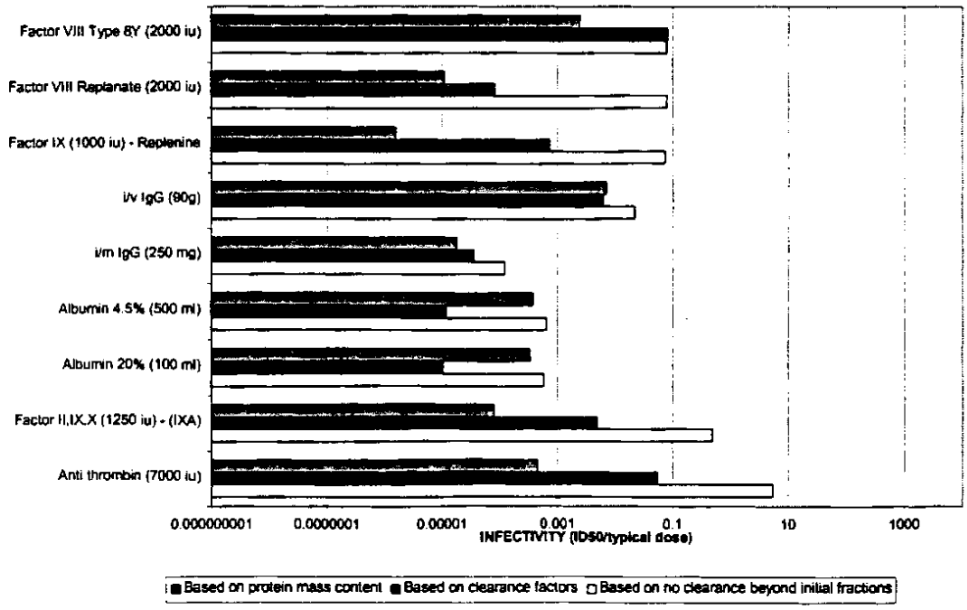
Table II.4.2 and Figure II.4.1 give the results from applying the above approaches to several key plasma derivatives. The results are expressed in the form of infectivity for typical dose units given to patients.

Table II.4.2: Comparison of Estimates of Infectivity in Plasma Derivatives

Plasma Derivative And Unit Size	Source	Based On Protein Content Approach (ID_{50}/typical dose)	Based On largest single Clearance Factors	Based on no Clearance beyond initial Fractionation
Factor VIII Type 8Y (2000 iu)	Cryoprecipitate	2.4×10^{-3}	0.08	0.08
Factor VIII Replanate(2000iu)	Cryoprecipitate & Fraction V	1.1×10^{-5}	8×10^{-5}	0.08
Factor IX (1000 iu) Rep	Cryosupernatant	1.6×10^{-6}	0.0007	0.07
I/v IgG (90g)	Fraction II	7.1×10^{-3}	0.006	0.02
I/m IgG (250 mg)	Fraction II	1.8×10^{-5}	0.00004	0.0001
Albumin 4.5% (500 ml)	Fraction V	3.8×10^{-4}	0.00001	0.0006
Albumin 20% (100 ml)	Fraction V	3.4×10^{-4}	0.00001	0.0006
Factor II,IX,X (1250 iu)	Cryosupernat	8.1×10^{-5}	0.005	0.5
Anti- Thrombin (7000 iu)	Cryosupernat	4.5×10^{-4}	0.055	5

Results based on infectivity resulting from 1 contaminated plasma donation per batch of 20,000.

Figure II.4.1 Comparison of Estimates of Infectivity in Plasma Derivatives



II.4.3.11 Discussion

It should be noted that at present the 3 approaches assess infectivity in BPL products only, as they are applied to BPL product data (protein content), product yields, product dose size, plasma batch data etc. Infectivity of specific SNBTS products will require assessment of SNBTS data once the agreed approach has been defined.

Also, the CF approach has used estimated CF in some cases where BPL processing is different to SNBTS, based on assessment of the differences between the processes. This is because at present CF data on BPL processing is not available.

Looking at the protein content approach, for example with Factor VIII 8Y, it can be seen that the infectivity is 2 orders of magnitude lower than the pessimistic approach of assuming no further reduction in infectivity load beyond the initial fractionation. This is consistent with the reduction in protein content, because Factor VIII 8Y contains only 1% of the protein present in Cryoprecipitate. This demonstrates how the protein content approach does reduce infectivity load significantly where there are significant reductions in protein load. Conversely for albumin, the two approaches provide very similar outcomes because there is effectively no reduction in protein load between Factor V and Albumin.

The high infectivity per dose obtained for Anti thrombin under the worst case scenario is due to the relatively high dose (7,000 iu), and the relatively low number of units produced pa.

It can be seen that for some derivatives there is little difference in the estimated infectivity between the three approaches, whereas for others the differences are more substantial. For

most of the derivatives, the protein content approach and the CF method are within one or two orders of magnitude.

Because of the uncertainty in estimating the infectivity present in the plasma derivatives, DNV welcome further discussion to improve the assumptions in the estimations and to provide guidance on which of the approaches is most justified on scientific grounds.

II.5 Incubation Periods

II.5.1 vCJD Cases

Very little is known about the incubation period (from original infection to development of clinical signs) of vCJD. It is not known for certain when the 23 cases were infected. However, it is likely that they were infected by beef products between the first recorded case of BSE in 1986 and the offal ban in 1989. The first vCJD cases appeared in 1994, suggesting that the shortest incubation period is between 5 and 8 years for transmission across the species barrier. For example, one of the cases that appeared in 1997 had eaten no meat for 11 years, suggesting that in this case infection was in 1986 or earlier, with an incubation period of at least 11 years. It is not possible to obtain a median incubation period from this data, because it is not known how many other people are still incubating the disease.

The incubation period might be inferred from other TSEs. Incubation periods for sporadic and familial CJD cannot be determined, as there is no identifiable point of infection. However, incubation periods can be estimated for other acquired TSEs as follows.

II.5.2 Kuru

The incubation period for Kuru, a human TSE that occurred in cannibal tribes of Papua New Guinea, ranged from 5 to over 40 years (SEAC 1994 p25). No sources have been identified for a more detailed distribution. There are still some cases of kuru appearing, and there are some people exposed to kuru who are still alive and therefore potentially incubating the disease. Hence the upper limit of the incubation period is greater than 40 years.

II.5.3 Iatrogenic CJD

The incubation period in iatrogenic cases of CJD has ranged from 15 months to 30 years, and varies according to the route of exposure (see Table II.5.1, from Ricketts 1997). [Other data supplied by Brown indicates more cases due to dura mater and human growth hormone, but unchanged mean incubation periods, which seems surprising.]

The incubation period is in general longer for more peripheral routes of exposure, as illustrated in the table. Hence the values for growth hormone, which was injected via intra-muscular or subcutaneous routes, are the most appropriate for infection from blood transfusion. These have a mean of 12 years and a range of 5 to 30 years.

More detailed data has been supplied by the Institute of Child Health on the 27 cases of CJD due to human growth hormone (HGH) that have occurred in the UK up to March 1998. Incubation periods are difficult to estimate because the treatment with HGH lasted several years (for these 27 cases, the treatment duration had a mean of 6.4 years and standard deviation 2.7 years). Also, the onset of clinical symptoms is not well defined, and the time when they are detected is influenced by the patient's knowledge of the risks. For the present study, incubation periods have been estimated from the mid-point of treatment to the reported onset of symptoms.

Table II.5.1 Incubation Periods for Iatrogenic CJD

Mode Of Infection	No Of Cases	Route Of Entry	Mean (+ Range) Of Incubation Period
Instrumentation			
Neurosurgery	4	Intracerebral	20 months (15 - 28)
Stereotactic EEG	2	Intracerebral	18 months (16 - 20)
Tissue transfer			
Corneal transplant	2	Optic nerve	17 months (16 - 18)
Dura mater transplant	25	Cerebral surface	5.5 years (1.5 - 12)
Tissue extract transfer			
Growth hormone	76	Hematogenous	12 years (5 - 30)
Gonadotrophin	4	Hematogenous	13 years (12 - 16)

The resulting incubation periods of these 27 cases have a mean of 14.3 years, a standard deviation of 3.3 years, and a range of 8 to 19 years.

Figure II.5.1 shows the incubation periods in relation to the time of onset of symptoms. The trend line shows that these are tending to increase, as cases with longer incubation periods continue to appear. Any further cases would be expected to have incubation periods longer than 13 years, since treatment with HGH stopped in the UK in 1985. The final mean value may be in excess of 15 years, but it is difficult at present to predict what it might be.

Figure II.5.1 Incubation Period Trend for UK HGH Cases

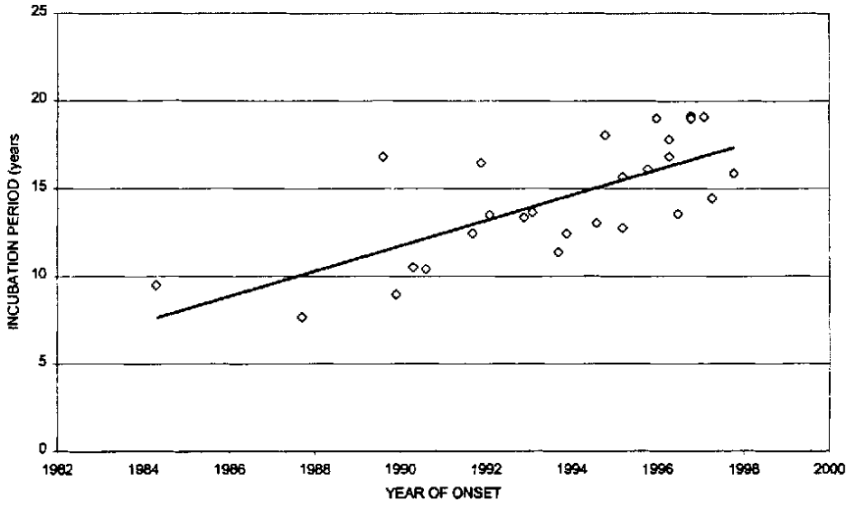
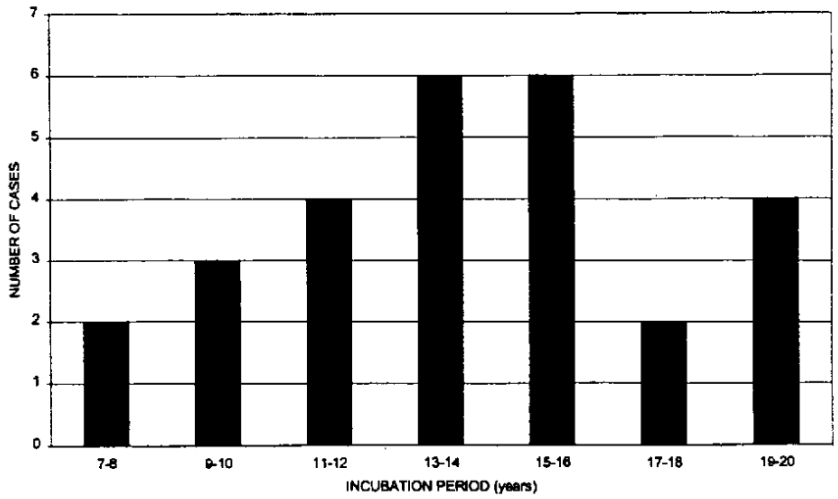


Figure II.5.2 shows the distribution of the incubation periods in the 27 cases. This has a peak around 15 years, but the higher incubation period end of the distribution, where cases are still appearing, is not well defined. Hence this does not show the shape of the final distribution.

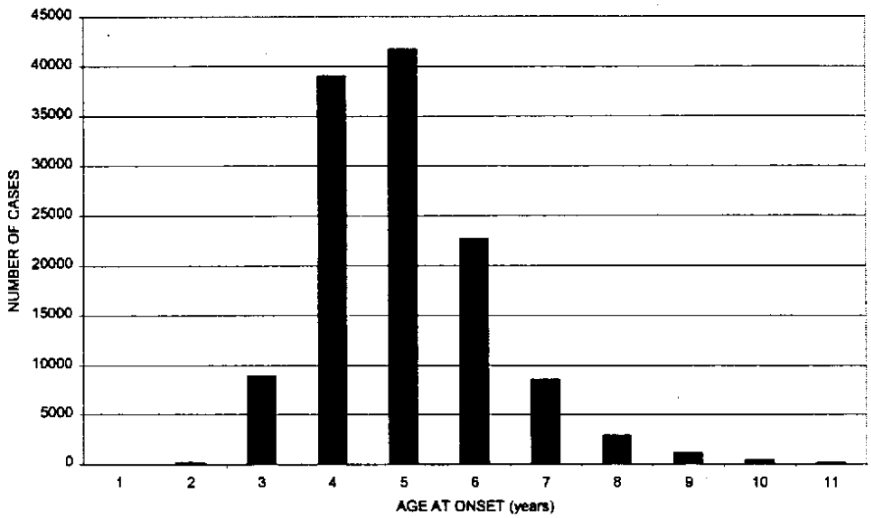
Figure II.5.2 Incubation Period Distribution for UK HGH Cases



II.5.4 BSE

The ages at onset of BSE in cattle have been supplied by MAFF for the 125963 cases of BSE reported up to January 1998. The mean is approximately 5 years with a 90% range of 3.5 to 7.5 years, i.e. 0.7 to 1.5x mean. The overall range is 20 months to 18 years (SEAC 1994 p35). Figure II.5.3 shows the distribution of the ages at onset, which has a peak around 4-5 years.

Figure II.5.3 Distribution of Age at Onset for BSE Cases

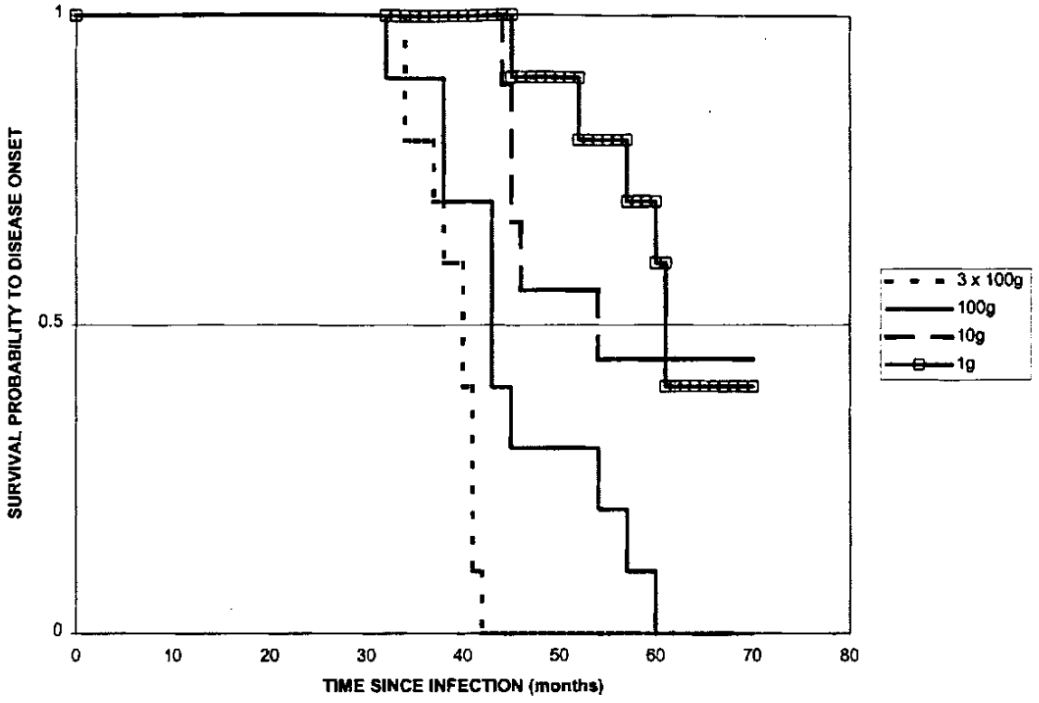


Most exposure to infected MBM was in calves, so the modal value may be appropriate for the incubation period. However, some adult cattle were also exposed, so the upper limit of the incubation period may be lower. The mean incubation period is usually assumed to be 5 years.

The distribution of the incubation period in cattle has been estimated from retrospective modelling of the BSE epidemic to be a gamma function with a standard deviation of 1.3 years, based on an assumed mean of 5 years (Anderson et al 1996). This gives a modal value of approximately 4.7 years, a 90% range of approximately 3 to 7.2 years (0.6 to 1.4x mean) and a 99% range of 2 to 9 years. This is consistent with the above data.

Oral challenge tests on cattle are still in progress. Interim results supplied by MAFF at March 1998 are shown in Figure II.5.4.

Figure II.5.4 Interim Results from Cattle Tests



Statistics on the incubation period based on cattle that have died so far are as follows:

3 x 100g dose	10/10 dead	Mean 3.2 years, standard deviation 0.2 years
100g dose	10/10 dead	Mean 3.8 years, standard deviation 0.7 years
10g dose	5/9 dead	Mean 3.9 years, standard deviation 0.3 years
1g dose	6/10 dead	Mean 4.7 years, standard deviation 0.5 years

The mean and standard deviation for the 10g and 1g tests will increase if further animals that are currently showing only early signs eventually die from the disease. In order to obtain estimates of the incubation period that will not be affected by subsequent deaths, the median and 10 percentile incubation periods are used, being the times at which at least 50% and 10% of the cattle had died. These are as follows:

3 x 100g dose	Median 3.3 years, 10% ile 2.8 years (0.82 x median)
100g dose	Median 3.6 years, 10% ile 2.7 years (0.74 x median)
10g dose	Median 4.5 years, 10% ile 3.7 years (0.81 x median)
1g dose	Median 5.1 years, 10% ile 3.8 years (0.74 x median)

This shows how the median incubation period has increased as the dose has reduced. The value for the 1g dose is very close to that estimated from the actual BSE cases. The spread of periods, as indicated by the 10 percentile as a fraction of the median, does not have any clear trend with dose.

II.5.5 Scrapie

The peak age of onset of scrapie in sheep is 3½ years (SEAC 1994 p27) although this is understood to vary with the sheep type. The incubation period in experimental intra-ocular inoculations was 14-22 months (SEAC 1994 p 33). However, maternal transmission is thought to be more likely, so a typical incubation period for natural scrapie would be about 3½ years.

The natural life expectancy of sheep is about 12 years if fed on a farm, although this reduces to 6-7 years if the animal is left to feed itself (according to the Meat & Livestock Commission). The typical incubation period for scrapie of about 3½ years is 30% of the former figure.

II.5.6 Application of BSE and Scrapie Data to Humans

The above estimates of incubation periods within different species may be used as an alternative approach to estimating human CJD incubation periods. However, since human life expectancy is much greater than cattle and sheep, it is desirable to investigate whether this might have any effect on the incubation period.

At one extreme, it is possible that the incubation period is an inherent property of each strain of TSE and route of infection, and that life expectancy has no effect. Hence the incubation period of vCJD in humans would be 5 years, as for BSE in cattle. This is consistent with the time between peak exposure to BSE infectivity through food (1992) and the occurrence of vCJD cases during 1996-7.

At the other extreme, it is possible that the incubation period is proportional to the natural life expectancy of the species. The mean incubation period for BSE in cattle of about 5 years is 17% of their natural life expectancy of about 30 years. Applying to humans, with a current life expectancy of about 77 years, this would indicate a mean incubation period of about 13 years. This agrees reasonably well with the data from CJD due to growth hormone, but is considered a less reliable approach.

More realistically, each combination of TSE strain and host genotype may yield distinctive incubation periods that cannot at present be predicted. For example, some TSE strain/mouse genotype combinations give incubation periods as low as 100 days, whereas other strains in the same genotype give incubation periods as high as 1000 days.

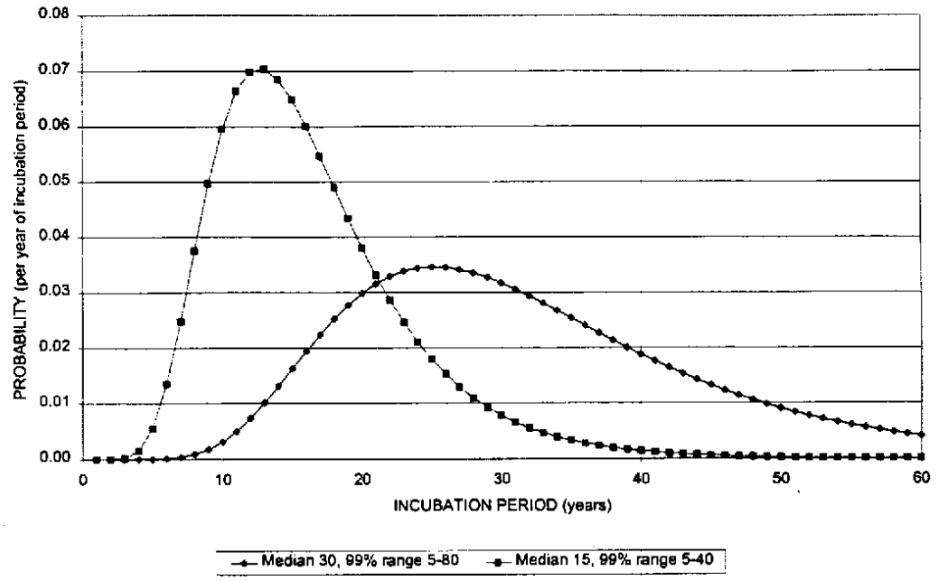
II.5.7 Previous Assumptions

Cousens et al (1997) assumed lognormal or gamma distributions with means in the range 10 to 25 years and 90%iles of 1.5x or 2x the mean.

II.5.8 Selection of Estimate for Infection from Blood

The HGH data is the best quality data on incubation periods of CJD, and being a peripheral route of exposure, it is most relevant to CJD from blood. However, the present data may under-estimate the incubation periods, because cases are still appearing. The UK data shows a mean incubation period of 14.3 years, which is increasing as cases continue to appear. Data on iatrogenic CJD from other countries and Kuru indicates a range of 5 to 40 years. These include at least 100 cases, and hence can be taken as a 99% range. This can be represented by a lognormal distribution with a median of 15 years and a 99% range 5 to 40 years, as shown in Figure II.5.5. This distribution has a mean of 16.3 years, a standard deviation of 7 years, and a 90% range of 7.5 to 30 years, or approximately 0.5 to 2x the mean.

Figure II.5.5 Assumed Incubation Period Distributions



II.5.9 Selection of Estimate for Infection from Food

Most of the above data involves infection within a species. For example, iatrogenic CJD involves infection with a human-passaged agent. This may be appropriate for vCJD from blood transfusion, but could be too short for vCJD obtained direct from BSE in food. When crossing a species barrier, the mean incubation period could be doubled, and the spread would be likely to increase. In order to model this for infections from BSE in food, a median of 30 years could be assumed, and a 99% range of 5 to 80 years. This distribution has a mean of 32.5 years, a standard deviation of 14 years, and a 90% range of 15 to 60 years, or approximately of 0.5 to 2x the mean. This is included in Figure II.5.5.

This distribution is consistent with the little available data on vCJD cases to date. However, it is highly speculative.

In addition, the incubation period might increase for the oral route, but available data on oral exposure of cattle to BSE does not support any further increase.

For sensitivity tests, the median could range from 15 to 60 years, with corresponding changes in the range.

II.5.10 Effect of Dose

The incubation period for scrapie is inversely related to the dose received. Regression analysis of scrapie in hamsters has established this relationship (Prusiner et al 1982). Hence it may be assumed that variations in dose account for much of the observed variation in incubation periods.

The attack rate experiments on cattle (Figure II.5.4) show similar trends, although the significance of the effect is relatively slight. An order of magnitude increase in dose produced only about a 10% reduction in the median incubation period.

If there was a large influence of dose on incubation period, it would be expected that the first cases of vCJD from food would have been people who had eaten bovine brain as a delicacy. Since this does not appear to be the case, it is consistent with the attack rate experiments in suggesting only a minor effect.

No information is known on how the average dose of infectivity from HGH might compare to the average dose from food or blood. However, it is likely that doses from blood transfusions could be very large, whereas doses from plasma derivatives could be very small. However, no model of these effects is adopted at present. It is assumed that the range of incubation periods accounts for variation in the doses received.

II.5.11 Effect of Genetic Factors

There is evidence that the nature of TSEs is affected by genetic factors, particularly in CJD in humans by the amino acid permutations (methionine/valine polymorphism) encoded by codon 129 on the PrP gene. In the UK population as a whole, 37% are homozygous for methionine at this locus (met/met); 11% are homozygous for valine (val/val) and 52% are heterozygotes (met/val) (Preece 1993).

So far, all cases of vCJD are homozygous for methionine at codon 129. This may indicate that only 37% of people are vulnerable to the disease. Alternatively, it may be that these people are more likely to have a short incubation period, and that others may be infected with a longer incubation period.

In sporadic CJD cases, 83% are homozygous for methionine. In iatrogenic CJD cases due to human growth hormone, 43% are homozygous for methionine (met/met); 47% are homozygous for valine (val/val) and 10% are heterozygotes (met/val) (Preece 1993). This suggests that the met/val genotype exerts some protective effect, but it is not complete. Such

people may be vulnerable to the disease with a reduced probability of infection or a longer incubation period.

In the present study, it is assumed that the range of incubation periods accounts for variations in genetic factors, and all people are assumed to be capable of being infected with vCJD.

II.6 Dose-Response Relationship

II.6.1 Possible Dose-Response Models

In this study, infectivity is measured in terms of ID_{50}, i.e. the dose that would cause infection of 50% of the population if administered to each. The relationship between the dose (measured in terms of multiples or fractions of ID_{50}) and the response (measured in terms of the probability of infection for an individual) is not well defined for TSEs.

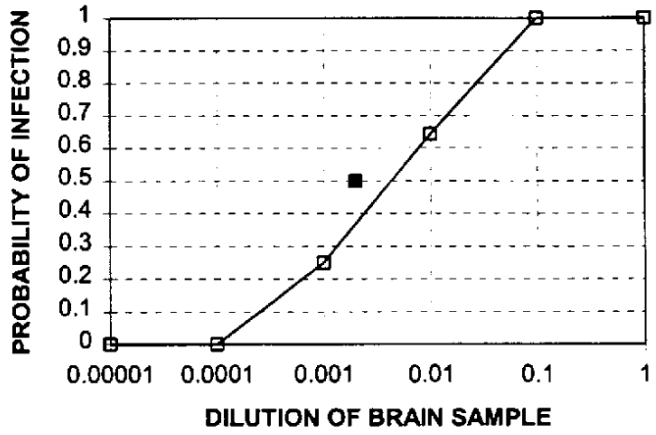
Smaller doses than the ID_{50} may cause deaths among some of the exposed population. This may be due to:

- Variation in individual susceptibility to infection.
- An element of chance in whether or not a given dose will infect a given individual.

A typical dose-response relationship is sigmoidal in shape, i.e. a plot of the probability of infection on a basis of log(dose) is S-shaped, resulting from a log-normal distribution of susceptibility in the population.

From data presented by Taylor et al (1995), a dose-response curve can be constructed for intraperitoneal injection of a relatively dilute pool of BSE infected brain into mice, as shown in Figure II.6.1.

Figure II.6.1 Dose-Response Curve for Mice Injected with BSE Infected Brain

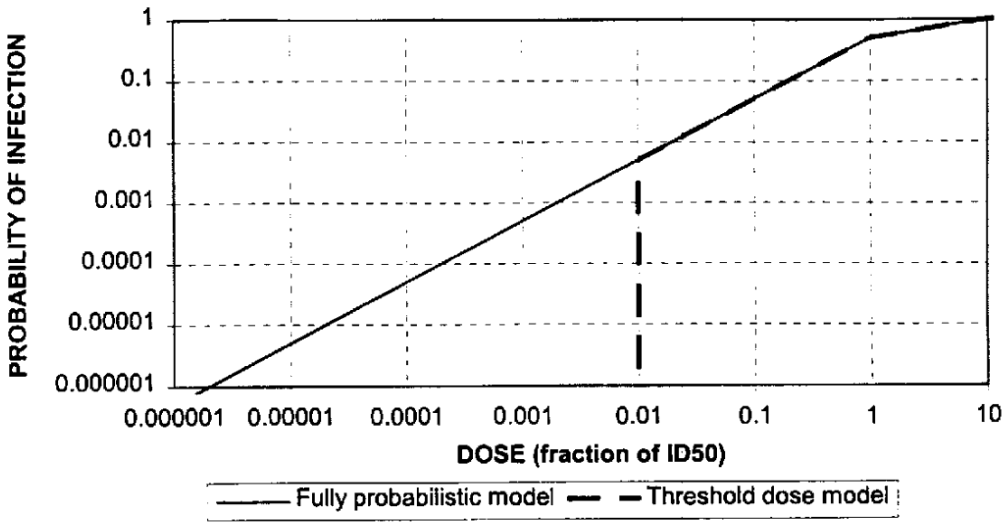


The tested populations were too small (approximately 13 mice in each group) to give precise estimates of the probabilities of infection from small doses, and the results are consistent with a more rounded sigmoid curve.

Various different forms of the dose-response relationship could be assumed for small doses. The simplest options are illustrated in Figure II.6.2:

- A linear dose-response (or fully-probabilistic) model, in which small doses have correspondingly small probabilities of infection. For example, if an ID_{50} has a probability of approximately 50% of causing infection of a given individual, a dose of 10^{-6} ID_{50} might have a probability of 0.5×10^{-6} of causing an infection. This linear dose-response relationship continues up to a dose of 2 ID_{50}, after which infection is regarded as certain. This approach will tend to overestimate the effect of small doses. This purely probabilistic dose-response model with no threshold has previously been used in quantifying risks from BSE via environmental pathways.
- A threshold dose model, assuming that small doses could exist which have no potential for causing infection. This would allow unlimited exposure to infectivity without infection, providing that the doses were always below some threshold value. This threshold model is a simple approximation to the more realistic sigmoid dose-response curve.

Figure II.6.2 Possible Dose-Response Models



II.6.2 Effect of Repeated Dosing on Infectivity

The studies of repeated dosing by Diringer et al (1998) do not give any information about the possible existence of a threshold dose, because the numbers of animals infected were too few (80 at most for any given dilution and feeding schedule). Demonstrating a threshold dose would require much larger groups of animals, preferably at dilutions of less than log steps. The rough correspondence between log dilution and log probability of infection in fact provides evidence for the linear dose model.

However, the studies do indicate that the dosing interval affects the probability of infection. In other words, a regular dose is less infective with longer intervals between individual doses. In addition, the cumulative effect of a regular dose is less than a single administration of the cumulative dose. If the latter effect appeared only at very low doses, it might indicate a

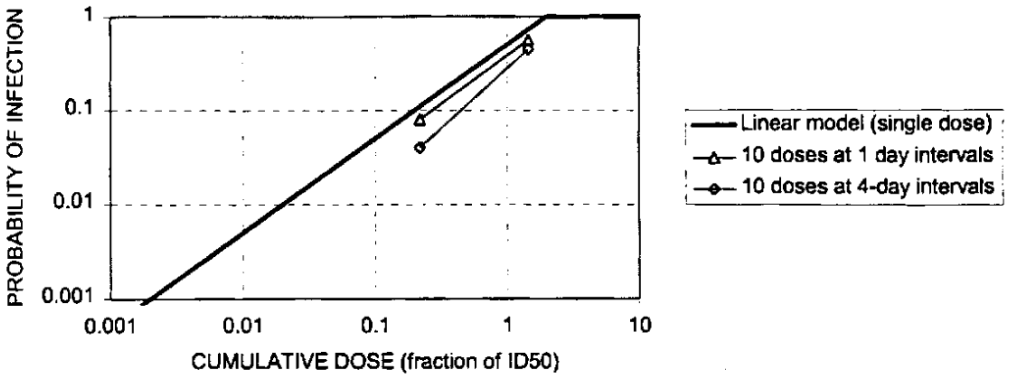
threshold, but in fact it appears across all doses where the probability of infection is less than 100%.

This issue is important when considering recipients of plasma products, who might receive regular doses that are individually very small but cumulatively significant.

Figure II.6.3 shows the dose-response relationship implied by the results from Diringer et al (1998) for 3 feeding schedules with the same cumulative dose:

1. A single dose D (results adjusted as described by Diringer et al from a single dose of D/10)
2. 10 equal doses of D/10 at 1-day intervals
3. 10 equal doses of D/10 at 4-day intervals

Figure II.6.3 Cumulative Dose Model



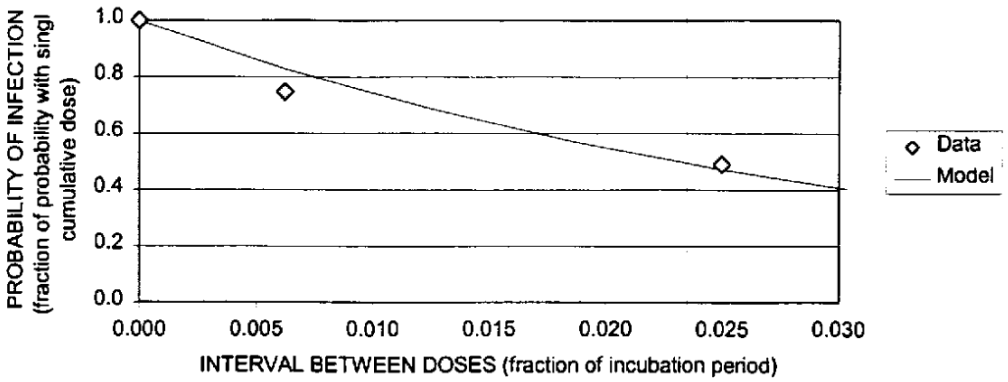
A suitable practical model of these results could convert a regular dose into an equivalent single dose, with the same probability of infection. Two key issues need resolution:

- How do 1-day and 4-day intervals for scrapie in hamsters relate to vCJD in humans?
- Over what period should a continuing regular dose be cumulated?

In the absence of any information on how to relate timescales for hamster scrapie to human vCJD, it is assumed that they scale in proportion to the incubation period of the disease. Hamster scrapie has an incubation period of about 160 days, depending on the dose (Diringer et al 1998). The median incubation period for human vCJD infection from blood is assumed to be 15 years (Section II.5.8). Hence 1 day for hamster scrapie is about 0.6% of the incubation period, and assumed equivalent to 34 days (i.e. about a month) for human vCJD. This is clearly very speculative, but is necessary to quantify the model.

Figure II.6.4 shows the average effect of dosing interval estimated from the results above. Dosing interval is expressed as a fraction of incubation period. A single dose (Schedule 1) is in effect a dosing interval of 0.

Figure II.6.4 Effect of Dose Interval for 10 Equal Doses



These results can be represented by a general model as follows:

$$D_e = D\, e^{-30\, T/I}$$

where:

D_e	=	equivalent single dose (ID50)
D	=	actual cumulative dose in time period (ID50)
T	=	mean interval between individual doses (years)
I	=	median incubation period (years)

The Diringer results only cover a course of 10 equal doses. What would be the effect of a continuing regular dose? This could be investigated by integrating the above model. A simpler approach is to neglect doses more than a year apart (in human vCJD terms), since the model shows that the effect of 10 doses a year apart is approximately 10% of the cumulative dose, i.e. roughly equal to that of the first dose on its own. This is again very speculative.

Hence the model can be expressed in terms of human vCJD as:

$$D_e = D_i\, R\, e^{-2/R}$$

where:

D_e	=	equivalent single dose (ID50)
D_i	=	individual dose (ID50)
R	=	dose rate (individual doses per year)

For example, a patient receiving a dose of 0.001 ID_{50} once per month for 20 years would have an actual cumulative dose of 0.012 ID_{50} per year, or a lifetime dose of 0.24 ID_{50}. The model above would indicate an effective dose of $0.012\, e^{-2/12} = 0.01$ ID_{50}.

This indicates that the effect of the above model is relatively small unless the individual receives continuing doses over several years. Then its effect is to ignore doses received after the first year.

II.6.3 Proposed Approach

DNV propose to continue with the assumption that the infectivity of each individual dose follows the linear dose-relationship, with no threshold dose. This is likely to be pessimistic, although no evidence is available to improve it.

DNV developed a model (detailed in Section II.6.2) to examine if there was any clearance of infectivity due to the time period between regular doses. The model indicated that risk is not significantly reduced (relative to the large uncertainties involved in this study) except for those individuals receiving continuing doses over several years. Hence, this study assumes that the cumulative effect of repeated doses over a one year period will be additive, and that no further risk is accumulated after 1 year.

II.7 Conclusions

The following conclusions are drawn about CJD in blood:

- Blood from humans with symptomatic CJD appears to contain infectivity at a relatively low level. Experiments on animals indicate that it is sometimes capable of causing infection, especially when inoculated intracerebrally into rodents. It is possible that these experiments are all flawed, but at present it is prudent to assume that human blood is infective for other humans.
- Experiments in several animals models have shown that blood from an animal infected with a TSE can be infective when inoculated intracerebrally into the same species.
- There has been a single report of a TSE being successfully transmitted by blood transfusion in an animal model. No human cases are known, although a few cases could have occurred without being detected.
- Blood from vCJD cases may be infective at a higher level than blood from sporadic CJD cases. This would make infection through blood transfusions more likely for vCJD.
- Evidence about the infectivity of blood from asymptomatic infections is unclear. At present, it is prudent to assume that it contains infectivity throughout the incubation period.

The following assumptions are made for quantitative modelling of the infectivity of vCJD in blood:

1. Infectivity of blood from vCJD cases is estimated to be 2 human i/v ID_{50}/ml human blood (based on tests on mice with CJD). The range (based on other animal experiments) could be 0.2 to 60 i/v ID_{50}/ml. Allowing for the possibility that blood is not infective, the range would be 0 to 60 i/v ID_{50}/ml.

2. Infectivity is assumed to be constant throughout the incubation period. It is also possible that it is higher at first but progressively declines through the incubation period, or alternatively that it is low at first but rises through the incubation period.

3. The incubation period for vCJD derived from blood is assumed to have a median of 15 years and a 90% range of 5 to 30 years (based on cases of CJD due to human growth hormone). For vCJD derived from BSE via food, it is assumed to have a median of 30 years and a range of 5 to 80 years (based on judgement).

4. Infectivity in blood components is assumed to vary from the value for whole blood above as shown in Table II.3.12, based on animal experiments by Brown et al (1998).

5. The infectivity in plasma derivatives has been assessed under three different scenarios. Firstly, by assuming the derivative has the same infectivity per gram of protein as in the plasma fraction from which they are made. Secondly, by assuming the derivative infectivity is reduced according to clearance factors. A worst case scenario of no infectivity clearance from processing has also been examined. For some derivatives there is little difference in the estimated infectivity between the three approaches, whereas

for others the differences are more substantial. For most of the derivatives, the protein content approach and the CF method are within one or two orders of magnitude. Because of the uncertainty in estimating the infectivity present in the plasma derivatives, DNV welcome further discussion on which of the estimates is most justified on scientific grounds.

6. Leucodepletion soon after donation is assumed to reduce the infectivity by 2 orders of magnitude compared to red cells with buffy coat removed (based mainly on judgement).

7. The dose-response function for vCJD infectivity is assumed to be linear with no threshold. A dose of 1 ID_{50} is assumed to give a 50% chance of infection, and smaller doses give proportionately smaller chances of infection. A dose of 2 ID_{50} or more is assumed to give certain infection. DNV propose that repeated doses over a one year period have an additive risk, and that there is no increase in risk beyond one year's repeated dosing.

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