RISK ANALYSIS OF NEW VARIANT
CREUTZFELDT-JAKOB DISEASE TRANSMISSION
BY BLOOD AND BLOOD PRODUCTS

RECOMMENDATIONS

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Agence Française de Sécurité Sanitaire des Produits de Santé (Afssaps)
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Introduction

This report summarizes the work of an independent multidisciplinary group of 16 experts (Annick Alpérovitch, Francis Barin, Bernard Bégaud, Sadek Béloucif, Thierry Billette de Villemeur, Paul Brown, Annette Bussel, Christian Conseiller, Roland Dobbelaer, Dominique Dormont, Marc Eloit, Jenny Goudemand, Yves Gruel, Norbert Ifrah, Patricia Ribaud et Michel Setbon) convened by the French Agency for Health Product Safety (Agence française de sécurité sanitaire des produits de santé; Afssaps) on Fridays 17 and 24 November 2000 and Monday 4 December 2000.

The experts were given three tasks:

- To review knowledge on the possible risk of transmission of new variant Creutzfeldt-Jakob disease (nvCJD) by blood,
- To assess all measures potentially capable of reducing this risk,
- If necessary, to make recommendations aimed at complementing, in an appropriate and proportionate manner, measures in place to guaranteeing the positive risk-benefit ratio of blood products.

Each individual product (labile blood products, LBP; and plasma-derived medicinal products, PDMP) was thus assessed on the basis of:

- Current measures,
- The possibility of introducing supplementary measures rapidly, taking into account both:
  - Their efficacy in reducing the hypothetical risk of transmission, and
  - Their limitations and possible drawbacks.

The present assessment, following on from the February 2000 report on the revision of risk-reduction measures, was based on all the available data (epidemiological data, scientific data on the risk of transmission, data on potential risk-reduction measures, and data on current use of blood products and alternatives).

1. Current knowledge

1.1 Evolution of the BSE and nvCJD epidemics

The number of cases of BSE diagnosed in France (approximately 190 since 1990) has continued to increase, whereas the United Kingdom epidemic peaked in 1992 (37 280 cases). However, the total number of cases identified in the United Kingdom is considerably higher (179 256 in October 2000).

Currently, three cases of the human disease, nvCJD, have been identified in France (these cases were already known in February 2000), compared to 85 (as of 06/11/00) in the United Kingdom, i.e. an incidence ratio of 28 (Poisson bilateral 95% confidence interval: 8 to 169).

1.2 Estimation of the number of persons likely to develop nvCJD

The model published on 20 August 2000 by Ghani et al (Anderson's team, Oxford) in Nature (10; 406: 583-4) yields an extremely broad estimate of the future number of cases in the United Kingdom, because it considers a vast number of scenarios (5 million), some of which, such as those based on a mean "incubation period" of more than 60 years, appear highly improbable. Dr Annick Alperovitch, by limiting the model of Ghani et al to more realistic hypotheses, made the following estimates of the number of cases in the United Kingdom:

- From 110 to 2 800 cases for a mean incubation period of 20 to 30 years, and
- From 150 to 6 000 cases for a mean incubation period of 30 to 60 years
On the basis of this last hypothesis (the most pessimistic) and a 20-fold lower level of exposure to the risk of BSE in France (based on consumption of bovine products imported from the UK, and the prevalence rates of nvCJD), the total number of cases in France would be 6 to 300 (a mean incidence of 5 diagnosed cases per year) if one considers the highest estimation corresponding to a mean incubation period of 60 years.

1.3. Presence of infectivity in blood

Presence of infectivity has never been demonstrated in a well-documented and reproducible manner in naturally occurring TSE cases, either in animals or in humans. In contrast, such infectivity has been proven in several experimental animal models.

Experimental work on nvCJD is underway in animal models to determine whether the infectious agent can effectively be detected in blood; the results are expected within a few months (transgenic mouse models) to a few years (primate models).

1.4. Estimation of the hypothetical infectious load in blood

There are several ways of expressing infectivity:

- One is the standard notion of the lethal dose 50 (LD50), i.e. the dose which, when administered by a given route (in this case, generally the intracerebral route) induces the disease in 50% of exposed animals (generally mice).
- Another is based on the infectious unit (Inf.U), defined as the minimal infectious dose capable of transmitting the disease to one experimental animal by a given route. Strictly speaking, this notion only applies if the entire sample is inoculated to the animals. This latter mode of expression was chosen for the present report, assuming that exposure to one or more infectious units may infect an individual. However, given the broad hypothesis used in the calculations, the conclusions should not be influenced by the use of one or other mode of expression.

Globally, it can be assumed that:

- The intravenous route is, on average, 10 times less infective than the intracerebral route (generally used as the reference). This means that, to induce the disease, it would be necessary to give a dose 10 times larger intravenously than intracerebrally, or that an infectivity of 100 Inf.U/ml when injected in the brain (Inf.U-ic) corresponds to an infectivity of 10 Inf.U/ml when injected in the blood (Inf.U-iv).
- As regards blood infectivity, in experimental models, 90% of the infectivity is associated with the buffy coat (mainly with leucocytes). The residual infectivity of plasma, after complete leucodepletion (including cell debris), can therefore be estimated at 1/10th that of whole blood.

Currently, there is no validated test capable of detecting the pathological protein in blood or blood products. Indeed, as stated above, the infectivity of whole blood would be no more than 100 intracerebral infectious units per millilitre (10 Inf.U/ml for fully leucocyte-depleted plasma). This corresponds to approximately one picogram of the pathological protein, which cannot currently be detected by methods such as western blotting, capillary electrophoresis and ELISA. None of the currently available methods are sufficiently sensitive, and none have been validated.

1.5. Possible existence of asymptomatic infected subjects

In August 2000, John Collinge's team published an article in PNAS showing that mice infected with a TSE strain isolated from another animal species (hamster) remained asymptomatic while their central nervous systems were infective (bioassay), but the infectious agent was undetectable (by physico-chemical tests). These findings support the hypothesis (already mentioned in the expert report dated February 2000) whereby humans might be permanently or transiently infected without developing the disease. The Expert Group considered it impossible to reliably estimate the number of subjects possibly involved.
1.6. Possible transmission of the disease by blood

There are currently no suspected or proven cases of nvCJD transmission to humans by blood or blood products, although this may be due to the recent nature of the new variant disease. Since the Expert Report of February 2000, Houston et al (Lancet, 16 September 2000) have published a case of BSE transmission from a sheep (experimentally infected by oral route with the BSE agent) to another apparently healthy sheep, after IV injection of 400 ml of whole blood collected halfway through the incubation period (while the animal was still asymptomatic). These results, which are in keeping with previous experimental studies in other species (mouse, hamster), therefore raise the possibility of intraspecies transmission of BSE by blood collected during the asymptomatic phase. These published results are preliminary, however, and the following points should be noted:

- Only one of 19 transfused sheep has so far developed the disease,
- Although highly probable, it has not yet been proven that the pathogenic agent in the recipient sheep was the same as that in the donor sheep. This is necessary before accepting the results of Houston et al as the first demonstration of intraspecies BSE transmission by blood transfusion (this work is ongoing).

In summary, the Expert Group considers that, while the possibility of TSE transmission by blood is in keeping with current data on experimental models, such transmission has never been reported for "natural" TSE (BSE in cattle, scrapie in sheep, and sporadic, familial or iatrogenic CJD in humans).

1.7. Quantified estimation of the risk in France

On the basis of the model mentioned in section 1.2, the maximum number of subjects likely to develop the disease in France over the next 60 years would be about 300. (During the same period, about 3 600 cases of sporadic CJD will be clinically diagnosed.)

Assuming that the blood of all these subjects, who are currently asymptomatic, is infective throughout the incubation period, the prevalence among the entire potential blood donor population (36 million subjects aged from 18 to 65) would be no more than 8.33 per million (1 per 120 000). Assuming that blood donors are a random sample of the French population (there are no data to contradict this assumption), a maximum of one blood donation per 120 000 could be infected. This latter estimation is used here as a worst-case hypothesis, given the lack of precise data.

The Expert Group thus used a pessimistic working hypothesis in which: 1) the infectious agent is present in human blood throughout the pre-clinical incubation phase, 2) that it can be transmitted by blood to other subjects, and 3) that the latter would develop the disease.

Measures capable of reducing this theoretical risk fall into two categories: general measures applying to all products, and specific measures applying to individual types of product.

2. General measures

2.1. Reduction in the target population of users

2.1.1. Strict respect of therapeutic indications

Given the possibility of a risk, albeit theoretical, this simple measure should be implemented first. It reduces the exposed population to only those patients imperatively requiring labile blood products (LBP) or plasma-derived medicinal products (PDMP). As already stated in the February 2000 report, the Expert Group re-iterates the urgent need for a campaign, targeting all health professionals, to promote strict respect of therapeutic indications for all LBP and PDMP, together with the most recent recommendations (e.g. those published by ANAES in 1997).

Concerning neonates and young children, techniques aimed at reducing exposure to the risk must be adopted whenever possible; this means, for example, using paediatric units of red cell concentrates, and dividing apheresis platelet concentrates (APC).
2.1.2. Development and use of alternatives to blood products

Such alternatives include synthetic products such as recombinant factor VIII, and the use of growth factors, such as erythropoietin to correct moderate anaemia (haemoglobinemia between 7 and 10 g/dl) in non-emergency settings. Autologous transfusion is another useful alternative.

For each type of blood product, the Expert Group examined (i) the existence and possible use of alternatives, (ii) likely progress in this field, and (iii) the advantages and disadvantages of each alternative.

2.2. Exclusion of certain donors

This measure essentially concerns persons having made long stays in a country with a high risk of exposure (mainly the United Kingdom between 1980 and 1996).

Most members of the Expert Group considered that the assessment presented in the February 2000 report was still valid concerning the relative levels of exposure to the risk of BSE in the United Kingdom and France, and endorsed the recommendation that persons having stayed for long periods in the United Kingdom between 1980 and 1996 should not be excluded from blood donation.

On the basis of a level of exposure 20 times higher in the United Kingdom, exclusion of donors having stayed there for 6 months or more during this period (approximately 1 to 2% of donors) would only reduce total exposure to the risk by approximately 3.8%.

Considering the small expected number of cases, this measure appears inefficient and disproportionate to the desired reduction in the risk at the population level.

However, some members of the Expert Group wished to draw the authorities’ attention to the fact that, at the individual level, a prolonged stay in the United Kingdom during the period 1980 to 1996 leads to a risk significantly higher than that in the general French population, and that donations by these subjects should be excluded, at least for the preparation of labile blood products, which are not subject to the reduction factors inherent in the purification steps applied to other PDMP.

Indeed, assuming a level of exposure 20 times lower in France than in the United Kingdom, stays in the United Kingdom between 1980 and 1996 would multiply the risk by a factor of 1.5 after 6 months; 2.1 after one year; 4.3 after 3 years; 12.2 after 10 years; and 20 after 17 years (the entire period). Considering the estimated risk in the French population, however, the increase in the risk would only become appreciable after a stay (or cumulative stays) exceeding one year.

It is noteworthy that exclusion of previously transfused subjects (a measure already in place), if effectively applied, solves the risk of secondary nvCJD transmission by blood products.

2.3. Importation

Importation of blood products could only be envisaged from countries:

- Where the risk of BSE is below that in France (based notably on the geographic classification of the European Scientific Steering Committee [GBR classification], and on imports of British bovine products),
- That are able to provide regular and adequate supplies. For example, while blood collection is currently decreasing in the United States, demand for fractionation is growing (especially demand from European countries, mainly the UK). This places strong supply-side pressure on high-quality plasma,
- That can guarantee quality and safety of products equal to that in France, in terms of donation ethics (voluntary, unpaid donors), donor selection, donation controls, traceability (hemovigilance), quality assurance, audits and inspection.

This measure would necessitate secure transport and cannot be applied to products with short shelf-lives such as platelet concentrates (5 days).
Moreover:

- It is difficult to be certain of the current risk of exposure to BSE in the country of procurement,
- A decision to import blood collected from paid donors would negatively impact French donors’ motivation, and this might have major public health repercussions.

2.4 Improvement of production processes

2.4.1. Leucodepletion

As, in experimental models of TSE, the infectivity associated with blood is mainly (90%) attributed to leucocytes, leucocyte depletion (further on referred to as leucodepletion) would significantly contribute to reducing the risk of nvCJD transmission by blood products. However, even total leucodepletion would not fully eliminate this theoretical risk, given the residual infectivity linked to plasma.

Leucodepletion, which has been applied to red blood cell and platelet concentrates since 1998, will be extended to all plasma produced in France by March-April 2001.

The Expert Group recommends (i) that this method be effectively generalized as rapidly as possible, (ii) that the efficiency of the leucodepletion process be regularly and routinely verified (counting residual cells) on a sufficient number of production units (the Expert Group would like producers to provide precise data on this quality control program) and, (iii) that non conform products be destroyed after the date of implementation of the measure (cf. (i)).

2.4.2. Nanofiltration (15 and/or 35 nanometers)

This process, which is only applicable to certain blood products (for reasons of molecular size and conformation), should in principle yield a very large reduction in the level of residual infectivity (for example, 5 logs, or a factor of 100,000, after filtration at 15 nanometers).

In the same way as for leucodepletion, the Expert Group recommends (i) that this process be applied as rapidly as possible, wherever possible, and (ii) that validation studies be undertaken for each product to which this measure is applied.

3. Analysis of the risk and specific recommendations for each blood product

Assuming that blood, the starting material, could be infected, the Expert Group envisaged separately the case of labile blood products and plasma-derived medicinal products, given the large differences in their mode of production, which might impact on the level of the theoretical residual infectivity.

Contrary to the risk of viral infection, for which reference data are available, estimations concerning nvCJD can only be based on hypotheses. The following pessimistic hypotheses were used when estimating the theoretical level of risk associated with the different blood products (cf. 1.4 and 1.6):

- In France, one in 120,000 blood donations could be infected,
- The infectious load in whole blood (calculated on the basis of intracerebral injection to mice) is 100 Inf.U-ic/ml (10 Inf.U-iv/ml in the case of intravenous injection),
- Leucodepletion reduces the infectivity of whole blood by a factor of 10,
- Residual infectivity of one or more Inf.U-iv per dose of finished product would make the product potentially infective when administered intravenously.

3.1. Labile blood products (LBP)

These are either cellular products (red cells and platelets) or fresh frozen plasma for therapeutic use. Approximately 2.6 million units of LBP were distributed in France in 1999; 85% were administered to subjects over 50 years of age and 60% to subjects over 70.
3.1.1. Cellular products

As previously mentioned, leucodepletion, introduced in 1998, is currently applied to all cellular products (red cells and platelets), the current standard being $10^6$ residual leucocytes per bag.

3.1.1.1. Red blood cell concentrates (RBCC)

These are obtained from a 450-ml unit of whole blood and represent 75% of annual LBP consumption (approximately 2 million units per year).

Calculations, based on the above hypotheses, show that an infected donation would yield a product that would remain potentially infective (residual infectivity exceeding one Inf.U-iv) even after leucodepletion of 3 logs. This residual infectivity is linked both to the persistence of leucocytes (approximately $10^6$ per bag) and to the plasma in the finished product; comparatively, the infectivity of red cells is negligible.

As each bag derives from a single donor, the theoretical risk of infection is therefore 1/120 000 per unit transfused, and increases proportionally with the number of units received. Thus, a patient receiving 5 RBCCs from 5 different donors would be exposed to a risk of 5/120 000, i.e. 1/24 000. The Expert Group underlines the fact that RBCCs are generally used in emergency or life-threatening situations in which the risk-benefit ratio (the risk remaining theoretical) is thus largely positive. Consequently, the Expert Group recommends the following measures:

- RBCC should only be used in indications in which the benefit is certain (life-threatening situations) and for which no alternative is available,
- Whenever possible, an alternative should be used, such as autologous transfusion, or erythropoietin (EPO) when the haemoglobin concentration is between 7 and 10 g/dl and the correction of anaemia is non urgent. The Expert Group also recommends that a task force be created to precisely re-assess the risk-benefit ratio of all such alternatives,
- Recommendations and good practice guidelines should be updated, if necessary, and circulated, as rapidly as possible, as part of an information campaign targeting health professionals,
- Producers should rapidly set up a study on the possibility of improving the efficiency of RBCC leucodepletion. An improvement of 1 log is feasible, and would further reduce the theoretical residual infectivity.

In contrast, the Expert Group considers that the following measures are either inappropriate or ineffective:

- Importation of RBCCs, for the reasons stated above (cf. 2.3); moreover, the mean shelf-life of red blood cell concentrates (42 days) would make this measure difficult to implement (although it would be materially possible),
- Further increasing the plasma content in RBCCs, given the already very small amount of residual plasma.

The Expert Group, for the reasons stated above (cf. 2.2), failed to reach a consensus on the exclusion of donors having stayed for long periods in the United Kingdom between 1980 and 1996.

3.1.1.2. Platelet concentrates

There are two types of platelet concentrate:

- Recovered platelet concentrate pools (RPCP) derived from 4 to 8 donors. In 1999, approximately 230 000 units of this product were used (200 to 500 ml per unit),
- Apheresis platelet concentrates (APC) obtained by selective platelet extraction from a single donor. In 1999, approximately 160 000 units of this product were used (200 to 600 ml per unit).
In the case where one donation is infected, the residual infectivity of the two types of preparation would exceed one infectious unit per bag. This residual infectivity is linked both to residual leucocytes (approximately $10^6$ per bag) and to plasma (comparatively, platelet-associated infectivity being negligible). From the calculation it appears that if one donation were infected, the infectious load in RPCP would be lower than that in APC; however, as 4 to 8 different donors contribute to one RPCP, the probability of getting one infected RPCP would be higher than for APC.

Taking into account the following facts: (i) that platelet concentrates carry a theoretical risk of nvCJD transmission, (ii) that the current indications of these concentrates concern approximately 70 000 patients, most of whom are elderly and have immediately life-threatening conditions, (iii) that there are currently no alternatives to these products (megakaryocyte growth factors are inappropriate in emergency situations), and (iv) that importation is materially impossible given the very short shelf-life of these concentrates (approximately 5 days), the Expert Group recommends the following measures:

- Use of these products should be restricted to those indications in which the benefit is indisputable (life-threatening conditions); APC should be preferred,
- Recommendations and good practice guidelines should be updated, if necessary, and circulated, as rapidly as possible, as part of an information campaign targeting health professionals,
- Producers should rapidly set up a study on the possibility of improving the efficiency of leucodepletion in platelet concentrates,
- Producers should rapidly set up a study on the possibility of reducing the volume of residual plasma in platelet concentrates.

3.1.2. Fresh frozen plasma (FFP)

There are two types of FFP, use of which is approximately equivalent (130 000 and 120 000 units distributed in 1999, respectively):

- Quarantined plasma (600-ml bags) derived from a single donor and subjected to a 120-day "quarantine",
- Viro-attenuated plasma (VAP) derived from a pool of 100 donations and subjected to a solvent-detergent treatment, active on enveloped viruses, followed by resin chromatography and several filtration steps (including a sterilization step at 0.2 µm).

Contrary to cellular products, leucodepletion is currently applied to only part of the FFP produced; this measure should be generalized to all FFP around April 2001.

Based on the same working hypotheses, the calculated residual infectivity in quarantined plasma, derived from one infected donation, would be above one Inf.U-iv per bag; as the plasma derived is from a single donor, the theoretical risk of getting one infected bag would be 1/120 000.

In the case of viro-attenuated plasma, the probability of infection of the initial pool is clearly higher (1/1 200, i.e. 8.3 per 10 000) owing to the larger number of donors (approximately 100). However:

- The infectious load may be diluted in the entire pool,
- The chromatography and filtration steps applied to this type of plasma introduce a major reduction factor (estimated at approximately 2 logs).

The estimated infectivity (0.02 Inf.U-iv per 200-ml bag) is far lower than that of quarantined plasma and largely below threshold of one infectious unit. However, this only holds true if the initial infectious load is diluted homogeneously throughout the pool. If this is not the case, the above value (0.02 Inf.U-iv per bag prepared from the pool) could also be interpreted as a probability of 0.02, i.e. 2% or 1/50, that a bag contains one infectious unit.

In this pessimistic hypothesis, based on the probability of 1/1 200 that a plasma pool would contain a donation from an infective donor, the probability that a bag would contain one infectious unit would be 1/1 200 x 1/50 = 1/60 000. Therefore, in theory, pooling, for the viro-attenuated plasma, does not increase the risk of contamination.
Furthermore, this product has a potential therapeutic advantage in some situations, together with a lower immunological risk (non erythrocyte-antigen specific antibodies) and a lower viral risk (the pools are screened for parvovirus by PCR). However, there is currently no firm evidence that one type of plasma is associated with a higher theoretical risk of infection by the nvCJD agent than the other.

Regarding fresh frozen plasma, the Expert Group thus recommends the following measures:

- Use restricted to indications in which the potential benefit is indisputable,
- Recommendations and good practice guidelines should be updated, if necessary, and circulated, as rapidly as possible, as part of an information campaign targeting health professionals; indeed, it appears that approximately 20% of administrations of fresh frozen plasma are not in keeping with current recommendations,
- Rapid application of the most efficient leucodepletion procedures to all such products. The efficiency of leucodepletion (residual leucocyte counts) must be verified routinely and regularly on an adequate number of production units,
- As soon as possible, the impact of the elimination steps on the residual infectivity of viro-attenuated plasma should be studied (for example, by experimental "spiking" with detectable infectious material), in order to determine the respective advantages of viro-attenuated and quarantined plasma regarding the theoretical risk of transmission of the nvCJD agent.

In contrast, given the low level of the risk and its theoretical nature, and for the reasons mentioned in section 2.3, the Expert Group considers that importation of fresh frozen plasma is inappropriate (despite the fact that, contrary to the previous two products, freezing prolongs the shelf life). Likewise, most of the experts do not consider the exclusion of certain donors to be warranted (cf. 2.2).

### 3.2. Plasma-derived medicinal products (PDMP)

The Expert Group examined the two following measures:

- Improving the industrial processes,
- Use of other products (raw materials such as plasma for fractionation, or finished products).

Currently, PDMP are produced from plasma for fractionation (pools of 300 to 8 400 litres derived from approximately 1 000 to 30 000 donations, according to the product). The annual requirements of the French Laboratory of Fractionation and Biotechnology (LFB, the French public establishment in charge of fractionation of the plasma collected in the French blood collection centres) are 520 000 litres per year. Currently, the level of residual leucocytes is approximately $10^4$ per bag in leucocyte-depleted plasma (50% of the plasma used for fractionation) and about $10^7$ per bag in the remaining 50%. By March-April 2001, all plasma for fractionation should contain only $10^3$ to $10^4$ leucocytes per bag.

PDMP manufacturing processes include elimination steps (alcohol fractionation, chromatographic columns, filtration) capable of reducing theoretical nvCJD infectivity.

Validation studies, based on spiking experiments with infectious material during steps mimicking the production process, can determine the residual infectivity after a given elimination step and, thus, a reduction factor for each step or the entire process.

The ensemble of elimination steps used during PDMP manufacturing processes generally lead to a very strong reduction in the initial infectious load (minimum 4 or 7 logs, for example). For each PDMP, calculations were made using two possible reduction factors (low and high), based on published data and validated fractionation data.

For some products, nanofiltration at 15 and/or 35 nm could reduce residual infectivity by further 4 or 5 logs. This nanofiltration is already applied to Factors IX and XI produced by the LFB; it should be extended to Factor VIII in early 2001, polyvalent immunoglobulins (TEGELINE°) in the second term of 2001, and von Willebrand factor in the fourth term of 2001.
For the different PDMP, theoretical residual infectivity was calculated by using the same hypotheses as for LBP, taking into account the following additional assumptions:

- The smallest plasma pool necessary for fractionation,
- The pool is infected by a single donation,
- The extraction yield,
- The cumulative reduction factor resulting from the manufacturing process, based on two possible reduction factors (high and low). For some products, this reduction factor is, or soon will be, increased by a nanofiltration step,
- The yearly total dose of product, administered continuously at the maximal dose regimen.

### 3.2.1. Factor VIII

This PDMP raises a particular problem, as hemophiliac patients are obliged to use Factor VIII repeatedly and at sometimes massive doses (up to 150 injections or 500,000 units per year).

Calculations, based on the pessimistic hypotheses mentioned above, suggest that, if a pool of plasma were infected, the residual infectivity per unit dose would be extremely low.

To calculate the cumulative risk theoretically incurred by a hemophiliac patient over a one-year period, the Expert Group considered the extreme case of a patient receiving 500,000 units of Factor VIII, all derived from infected plasma pools (a highly improbable worst-case scenario: on the basis of a population risk of 1/120,000, only one pool in 5 or 8 would be infected).

This hypothetical patient would be exposed to:

- 0.024 Inf.U-iv (-1.62 logs), assuming a global reduction factor of 4 logs during the manufacturing process, on the basis of one year of treatment at the maximal dose regimen, all the units coming from infected plasma pools,
- 0.000024 Inf.U-iv (-4.62 logs), assuming a global reduction factor of 7 logs.

Even in the pessimistic conditions, the level of exposure for a one-year treatment would be far below one infectious unit (by a factor of more than 40).

The above model assumes that the infectious load of the infected donation is homogeneously distributed throughout the initial pool. In this situation the yearly maximal dose, even if given in a single administration, would not be infective, the load being far lower than one infectious unit.

In another interpretation, the infectious doses, below one Inf.U-iv would not be fractionable. This would mean that the 0.024 Inf.U-iv should be considered as 2.4 Inf.U-iv distributed among the equivalent of 100 total yearly doses, potentially infecting 2.4% of patients treated annually at a total dose of 500,000 IU or one patient every 42 years.

In the specific case of Factor VIII, the Expert Group ruled out:

- Use of imported plasma for the preparation of Factor VIII, for the same reasons as those given in section 3.1.2.,
- Exclusion of certain donors, for the reasons given in section 2.2.

In contrast, the Expert Group considered two alternatives to Factor VIII produced from French plasma, namely recombinant Factor VIII and imported Factor VIII:

- Recombinant Factor VIII (which is not obtained from plasma and is therefore, in principle, devoid of any risk of nvCJD infection) currently represents 80% of all Factor VIII used in France (albeit with strong regional variations). One disadvantage of recombinant factors may be a higher incidence of anti-Factor VIII antibodies (inhibitors). This risk is higher in previously untreated patients (PUPs), and therefore particularly concerns young children. Situations can arise that are extremely difficult to manage in clinical practice.
On the basis of available data (published literature, National Hemophiliac Follow-Up Register) it is impossible to conclude that the incidence of inhibitors is higher with recombinant factors than with plasma-derived Factor VIII, but the Expert Group considers that this risk must be taken seriously, particularly in young children, and that, if there is a difference, it would be unfavourable to the recombinant factor. This risk justifies maintaining access to plasma-derived Factor VIII.

- Imported Factor VIII (extracted from plasma collected in countries which, in principle, have a lower risk of BSE) (see section 2.3). The supply of these factors appears to be adequate. It should, however, be noted that some of these factors, obtained by selective extraction of Factor VIII and lacking Willebrand Factor, and thus could induce a higher frequency of inhibitors than French plasma-derived Factor VIII.

The Expert Group also noted that, within a few weeks, Factor VIII produced by LFB will be nanofiltered, reducing by 4 to 5 logs (a factor of 10,000 to 100,000) any residual infectivity, which would thus become negligible, even in the most pessimistic hypothesis.

In conclusion, the Expert Group considers that, although the theoretical infectious load of French plasma-derived Factor VIII is, even in the most pessimistic scenario, very low, temporary alternatives, free of this risk, are available (recombinant Factor VIII and imported plasma-derived Factor VIII). However, the Expert Group considers that it would be inappropriate to impose a single therapeutic option, and that each patient should be able to choose freely on the basis of information provided by his/her doctor on the relative advantages and disadvantages. The Expert Group recommends that imported plasma-derived Factor VIII be made available as rapidly as possible. The Expert Group also recommends that LFB produce nanofiltered plasma-derived Factor VIII as soon as possible, thereby vastly improving the safety margin regarding the risk of nvCJD. When available, this nanofiltered Factor VIII should be used instead of standard Factor VIII. Moreover, the Expert Group considers, for the reasons mentioned above (risk of inhibitors) that the "recombinant only" option is inappropriate.

### 3.2.2. Other plasma-derived medicinal products (PDMP)

Table I summarizes estimated theoretical residual infectivity for the different PDMP marketed by LFB, in the hypothesis of starting from infected plasma pools.

These estimates are based on the same hypotheses and the same calculations as for Factor VIII, taking account of the reduction factor and the yield of each manufacturing process, and on the basis of continuous treatment for one year at the highest dose. These working hypotheses (unrealistic for several products) were adopted to obtain worst-case estimates and to provide a common basis on which to compare the different drugs.

The results are expressed in Inf.U-iv equivalents per maximal total yearly dose. The values given are either the mean infectious dose administered per patient (values below 1 corresponding to a zero risk of transmission) or the yearly probability of infecting one patient (see sections 3.1.2 and 3.2.1).

The Expert Group stresses the fact that these figures, although based on calculation methods validated in other situations (viral risk) are, in the present cases, only indicative, owing to the large number of assumptions required. As in the case of Factor VIII, two reduction factors (one low, one high), yielded by the manufacturing process were considered.

On the basis of the levels of residual infectivity considered above, the following PDMP present a theoretical risk of transmission:

- Factor VII,
- Fibrinogen,
- Fibrin glue
- Antithrombin III

It should, however, be borne in mind that this is an extremely pessimistic estimation, based on i) the smallest reduction factor, ii) on one year’s continuous use of the highest dose, and iii) every dose being derived from an infected plasma pool.
As fibrin glue is no longer marketed in France at the time of writing, and as alternative therapies are available, the Expert Group's recommendations will concern the following three drugs: factor VII, fibrinogen and antithrombin III.

3.2.2.1. Factor VII

The residual infectivity is lower than that in plasma-derived Factor VIII. However, if Factor VII of satisfactory quality, produced from plasma collected in countries where the risk of BSE is, in principle, lower (see section 2.3) is available, its importation should be authorized.

Use of Factor VII must be restricted to the sole indications in which the risk-benefit ratio is indisputable, i.e. life-threatening conditions.

3.2.2.2. Fibrinogen

Although residual infectivity is lower than that of the other three products, the use of fibrinogen should also be restricted to the sole indications in which the risk-benefit ratio is indisputable, i.e. life-threatening conditions. However, courses of fibrinogen are often short, thereby reducing the theoretical risk of transmission. In indications requiring repeated administration, if fibrinogen of satisfactory quality and produced from plasma collected in countries where the risk of BSE is, in principle, lower (see section 2.3) is available, its importation should be authorized (see section 2.3).

3.2.2.3. Antithrombin III

The Expert Group considers that the theoretical risk is similar to that associated with Factor VII and, consequently, that the same considerations apply.

In particular, if antithrombin III, of satisfactory quality and produced from plasma collected in countries where the risk of BSE is, in principle, lower (see section 2.3) is available, its importation should be authorized (see section 2.3).

3.2.2.4. Other plasma-derived medicinal products

Regarding the other plasma-derived medicinal products (e.g. albumin for therapeutic use, and immunoglobulins), the Expert Group considers that the level of risk (extremely low, especially in short-term use) does not warrant any particular recommendations.

Albumin is also used as an excipient in a number of medicinal products and other preparations. The amounts used are considerably lower than in therapeutic indications, thereby further reducing the theoretical risk of transmission to practically incalculable levels.
Table 1
Plasma-derived medicinal products: theoretical residual infectivity per recipient and per year, based on worst-case assumptions.

<table>
<thead>
<tr>
<th>Plasmatic Product</th>
<th>Low hypothesis</th>
<th>High hypothesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII</td>
<td>$2.4 \times 10^{-2}$</td>
<td>$2.4 \times 10^{-5}$</td>
</tr>
<tr>
<td>Factor VII</td>
<td>$1.5 \times 10^{-3}$</td>
<td>$1.5 \times 10^{-6}$</td>
</tr>
<tr>
<td>Factor IX</td>
<td>$7.2 \times 10^{-7}$</td>
<td>$7.2 \times 10^{-9}$</td>
</tr>
<tr>
<td>Factor XI</td>
<td>$3.5 \times 10^{-8}$</td>
<td>$3.5 \times 10^{-8}$</td>
</tr>
<tr>
<td>Willebrand Factor</td>
<td>$10 \times 10^{-7}$</td>
<td>$10 \times 10^{-8}$</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>$2 \times 10^{-4}$</td>
<td>$2 \times 10^{-5}$</td>
</tr>
<tr>
<td>PPSB</td>
<td>$1.9 \times 10^{-5}$</td>
<td>$1.9 \times 10^{-8}$</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>$2.7 \times 10^{-3}$</td>
<td>$2.7 \times 10^{-5}$</td>
</tr>
<tr>
<td>Protein C</td>
<td>$10 \times 10^{-9}$</td>
<td>$10 \times 10^{-9}$</td>
</tr>
<tr>
<td>Albumin</td>
<td>$7 \times 10^{-5}$</td>
<td>$7 \times 10^{-10}$</td>
</tr>
<tr>
<td>Alpha 1 antitrypsine</td>
<td>$4.4 \times 10^{-6}$</td>
<td>$4.4 \times 10^{-9}$</td>
</tr>
<tr>
<td>Polyvalent Immunoglobulins</td>
<td>$2.8 \times 10^{-5}$</td>
<td>$2.8 \times 10^{-10}$</td>
</tr>
<tr>
<td>Anti Hobs IV immunoglobulins</td>
<td>$1.5 \times 10^{-5}$</td>
<td>$1.5 \times 10^{-10}$</td>
</tr>
<tr>
<td>Anti HBs IM immunoglobulins</td>
<td>$9.5 \times 10^{-10}$</td>
<td>$9.5 \times 10^{-13}$</td>
</tr>
<tr>
<td>Anti D immunoglobulins</td>
<td>$1.5 \times 10^{-7}$</td>
<td>$1.5 \times 10^{-10}$</td>
</tr>
<tr>
<td>Antitetanic immunoglobulins</td>
<td>$9.5 \times 10^{-10}$</td>
<td>$9.5 \times 10^{-13}$</td>
</tr>
<tr>
<td>Thrombin glue (no longer marketed)</td>
<td>$5.4 \times 10^{-6}$</td>
<td>$5.4 \times 10^{-6}$</td>
</tr>
<tr>
<td>Fibrin glue (no longer marketed)</td>
<td>$5.5 \times 10^{-5}$</td>
<td>$5.5 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Results are expressed in Inf.U-iv equivalents per maximal total yearly dose. The values shown are either the mean infectious dose administered per patient (values below 1 corresponding to a zero risk of transmission) or as the annual probability of infecting one patient. In the case of Willebrand Factor, for example, the value of $10 \times 10^{-7}$ can be interpreted in two ways:

- As a dose far below 1 infectious unit, and therefore not infective,
- As a probability of infecting approximately one in 100 million patients (low hypothesis) treated continuously, for one year, at the maximal dose.
Conclusion

In the current state of scientific knowledge, it is impossible to know whether or not there is a risk of nvCJD transmission by blood and blood products in human. The existence of such a risk assumes i) that blood, from an infected but asymptomatic subject, is infectious; ii) that the infected subject gives blood during this incubation period; and iii) that the resulting infectivity is sufficient, when administered by the intravenous route, to infect the recipient.

However, identification of a marker of infectivity in lymphoid tissue of patients with nvCJD, and work done on animals with other strains of NCTA (non conventional transmissible agents), suggest that the risk of transmission cannot be ruled out. For this reason, precautionary measures are recommended (given the theoretical nature of the risk, the word prevention is inappropriate), given the potentially severe consequences for public health.

From current epidemiological data on nvCJD, one can estimate that no more than 6 to 300 persons will develop this disease in the next 60 years in France. This upper estimate (300) was used to calculate the theoretical risk that a blood donation be collected from a donor in the incubation period: 1 per 120 000 donations (this does not take into account the possibility of carriers, who would remain asymptomatic throughout life). Calculation of the theoretical residual infectivity of an LBP- or PDMP-unit, also required the infectious load of the donor’s blood to be postulated, on the basis of experimental animal data.

Finally, high and low hypotheses on the capabilities (reduction factors) of the different steps in the preparation of LBP or PDMP, to eliminate the infectious agent, were used to estimate the residual infectivity. Thus, the theoretical residual infectivity values given for each product in this report must be considered as indicative (and probably maximal), and not as absolute values. Nevertheless, although the absolute values are imprecise, the risk hierarchy they give for the different blood products is no doubt more robust.

Based on this risk analysis, blood products fall into two risk categories: labile blood products (LBP) and plasma-derived medicinal products (PDMP). Considering the infectivity hypotheses used here, LBP appear to be more at risk, as their preparation process cannot guarantee the safety of the final product if the initial donation was infected. However, these products are generally used in life-threatening situations, and there are no practical alternatives at present. As regards PDMP, manufactured from plasma, they undergo, during fractionation, a number of steps that increase their safety.

None of the PDMP has been judged as bearing a risk that would warrant its withdrawal. However, measures are recommended for each category of product to improve their safety margin, and alternative supply sources are proposed in some cases.
ANNEX 1 - Summary of the Expert Group’s recommendations

The Expert Group reached a consensus on the following measures:

**Labile blood products (LBP)**

*All products*
- Strict respect of indications for the use of LBP (restricted to life-threatening situations),
- If necessary, update of the recommendations on the use of blood products, which should then be widely communicated, especially to all health professionals,
- Leucodepletion must be extended to all products.

*Red blood cell concentrates (RBCC)*
- Use of alternatives whenever possible,
- Creation of a task force to reassess the risk-benefit ratio of alternatives to RBCC

*Platelet concentrates (APC, RPCP)*
- Reduction in the amount of plasma in platelet concentrates,
- Preferential use of platelet concentrates derived from single donors (APC)

*Fresh frozen plasma*
- Despite the pooling step, viro-attenuated plasma (VAP) does not carry a higher risk of nv-CJD than fresh frozen plasma (FFP),
- A study of the relative advantages of VAP and FFP, especially in the light of other quality criteria (viral risk, immunological risk) must be set up as soon as possible. For VAP, contribution of the production process to reduce the theoretical residual infectivity, should also be taken into consideration.

**Plasma-derived medicinal products**

Leucodepletion from $10^7$ to $10^4$ residual leucocytes per bag of plasma for fractionation seems to yield a marginal safety benefit. This measure must, however, be extended, especially for all such PDMPs with the smallest reduction factors during fractionation.

*Factor VIII*
- Imported plasma-derived Factor VIII must be made available immediately,
- Nanofiltered plasma-derived Factor VIII (LFB) must be made available as soon as possible, after which standard Factor VIII must be replaced by nanofiltered Factor VIII,
- In the meantime, no single therapeutic option (imported, recombinant or French plasma-derived Factor VIII) should be imposed; the choice must be left to the patient and physician. In particular, plasma-derived Factor VIII must remain available.

*Factor VII and antithrombin III*
- If products with satisfactory quality and derived from plasma collected in countries where the risk of BSE is in principle lower (see section 2.3) are available, they should be imported rapidly to permit a free choice.

*Fibrinogen*
- In indications necessitating prolonged use, fibrinogen of satisfactory quality and derived from plasma collected in countries where the risk of BSE is in principle lower, if available (see section 2.3), should be imported.

The Expert Group failed to reach a consensus on the question of recommending exclusion of donors having stayed for long periods in the United Kingdom during the period 1980-1996. In the opinion of some members of the Expert Group, exclusion of these donors might be warranted in light of the individual risk, particularly to reduce the theoretical risk of nvCJD transmission by labile blood products.
ANNEX 2 - Glossary

Afssaps:
Agence française de sécurité sanitaire des produits de santé (French Agency for Health Product Safety)

Leucodepletion:
Aseptic removal of most leucocytes from a labile blood product. For technical reasons, this process is usually incomplete; the term leucoreduction would thus be preferable.

BSE:
Bovine Spongiform Encephalopathy

TSE:
Transmissible Spongiform Encephalopathies

European CSD:
Scientific Steering Committee of the European Commission, Directorate Sanco. The committee has proposed a geographic classification of the risk of BSE (on a scale of I to IV).

PDMP:
Plasma-derived medicinal products: medicinal products, prepared industrially, from human plasma (albumin, clotting factors, immunoglobulins, etc.).

CJD:
Creutzfeldt-Jakob disease (sporadic, iatrogenic, familial)

nvCJD:
New variant clinical form of the Creutzfeldt-Jakob disease

LBP:
Labile blood products: whole blood, red cell concentrates, platelet concentrates, fresh frozen plasma and plasma for fractionation.

PrPres:
Or PrPsc, an abnormal form of the natural protein PrPc

Inf.U:
Infectious unit

IU:
International unit