

**FOOD AND DRUG ADMINISTRATION**  
**DEPARTMENT OF HEALTH AND HUMAN SERVICES**

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**Meeting of:**

**TRANSMISSIBLE**

**SPONGIFORM ENCEPHALOPATHIES**

**ADVISORY COMMITTEE**

**September 18, 2006**

**Holiday Inn Gaithersburg  
Gaithersburg, Maryland**

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P R O C E E D I N G S (8:30 a.m.)

**Agenda Item: Administrative Remarks.**

DR. FREAS: Good morning, and welcome to the 19th session of the transmissible spongiform encephalopathy advisory committee. I am Bill Freas. I am the executive secretary for today's meeting.

As announced in the Federal Register, and amended in the Federal Register, today's meeting and tomorrow's meeting are open to the public and the public is welcome to attend both days.

At this time, I would like to go around the head table and introduce the committee members to the public. Would the members please raise their hand when I call their name. I will be starting at the right side of the room at the audience's right.

In the first chair is Dr. Sue Priola, senior investigator, laboratory of persistent and viral diseases, Rocky Mountain Laboratories.

The next chair is empty right now, but it will soon be occupied by Dr. Michael Geschwind, assistant professor of neurology, University of California, San Francisco Medical Center.

The next chair is our consumer representative, Ms. Florence Kranitz, president of the CJD Foundation, Akron, Ohio.

The next chair is empty and that will be soon occupied by a new committee member, Dr. Laura Manuelidis, professor and head of neuropathology, Yale University School of Medicine.

Next we have David Gaylor, president of Gaylor Associates, Eureka Springs, Arkansas.

Next we have our industry representative, Dr. Taryn Rogalski-Salter, director of U.S. regulatory policy, Merck Research Laboratories.

Next we have Dr. Nick Hogan, associate professor of ophthalmology, University of Texas Southwestern Medical School.

Next we have Dr. Mo Salman, professor and director, animal population health institute, Colorado State University.

Around at the head of the table is our chair, Dr. Glenn Telling, associate professor, department of microbiology, immunology and molecular genetics, University of Kentucky.

Next we have Ms. Jan Hamilton, advocacy director, Hemophilia Foundation of America.

Next is another new member to this committee, Dr. Mark Powell, risk scientist, office of risk assessment and cost benefit analysis, U.S. Department of Agriculture.

Around the corner of the table we have Dr. James

Lillard, associate professor of microbiology, Morehouse School of Medicine.

In the next chair we have Dr. Lynn Creekmore, regional epidemiologist, AFIS veterinary services, U.S. Department of Agriculture.

Next we have Dr. James Sejvar, neuroepidemiologist, division of viral and rickettsial diseases, Centers for Disease Control and Prevention.

Next we have Mr. Val Bias, co-chairman, blood safety working group, National Hemophilia Foundation, Oakland, California.

Next we have another new member, Dr. Ronald Brookmeyer, professor, department of biostatistics, Bloomberg School of Public Health, Johns Hopkins University.

Next, Dr. Susan Leitman, chief, blood services section, department of transfusion medicine, National Institutes of Health.

Next is another new member, Dr. James Mastrianni, assistant professor of neurology, University of Chicago.

Next we have Dr. Richard Colvin, center for immunology and inflammatory diseases, Massachusetts General Hospital.

The empty chair will soon be filled by Dr. Richard Johnson, professor of neurology, Johns Hopkins

University.

Dr. Bernardino Ghetti could not be with us at today's meeting. I would like to welcome everyone else and thank you for coming.

Now I would like to read the conflict of interest statement into the record. The Food and Drug Administration is convening today's meeting of the transmissible spongiform encephalopathies advisory committee under the Federal Advisory Committee Act of 1972.

All members of the committee are special government employees or regular federal employees from other agencies, and are subject to federal conflict of interest laws and regulations.

The following information on the status of the committee's compliance with the federal conflict of interest laws, including but not limited to, 18 US Code 208 and 21 US Code Section 355(n)(4) is being announced in today's meeting and will be part of a public record.

FDA has determined that members of the committee are in compliance with the federal ethics and conflict of interest laws, including but not limited to 18 US Code section 208, and 21 US Code section 355(n)(4).

Under 18 US Code 208, applicable to all government agencies, and 21 US Code 355(n)(4), applicable to certain FDA committees, congress has authorized FDA to

grant waivers to special government employees who have financial conflicts when determined by the agency's need for that particular individual's services outweighs his or her potential conflict of interest, section 208, and where participation is necessary to afford essential expertise, section 355.

Members of the committee, including consultants, appointed as temporary voting members, appointed as temporary voting members, are special government employees or regular federal employees.

They have been screened for potential conflicts of interest of their own as well as those imputed to them including those of their employer, their spouse, minor child.

For the discussion topics, topic one, which is experimental clearance of transmissible spongiform encephalopathy infectivity in the plasma derived factor VIII products, and topic two, which is possible criteria for approval of donor screening tests for vCJD, these interests may include consulting, expert witness, testimony, contracts, grants, CRADAs, teaching, speaking, writing, patents and royalties and primary employment.

Today's agenda topics are considered general matters discussions. In accordance with 18 US Code Section 208(b)(3), general matters waivers have been granted to the

following:

Drs. Ronald Brookmeyer, Michael Geschwind, Bernardino Ghetti, James Lillard, Laura Manuelidis, and James Mastrianni.

Previously approved waivers for Mr. Val Bias, Dr. Lynn Creekmore, Dr. Nick Hogan, Ms. Florence Kranitz, Dr. Glenn Telling, Dr. Mo Salman, are in effect for this meeting.

A copy of the written waiver statement may be obtained by submitting a written request to the agency's freedom of information office, Room 12-A-30, of the Parklawn Building.

Dr. Taryn Rogalski-Salter is serving as the industry representative acting on behalf of all related industries and is employed by Merck Laboratories. Industry representatives are not special government employees and they do not vote.

With regard to the FDA's guest speakers, the agency has determined that information provided by these speakers is essential.

The following information is made public to allow the audience to objectively evaluate any presentations and comments made by the speakers for topic one:

Dr. Lisa Ferguson is employed by USDA in Hyattsville, Maryland. Dr. Jiri Safar is associate

professor, University of California, San Francisco. He has a financial interest in a company that is developing prion diagnostic products. As guest speakers, they will not participate in the committee deliberations, nor will they vote.

In addition, there are regulated industry and outside organization speakers at today's meeting making presentations.

These speakers may have financial conflicts of interest associated with their employer and with other regulated firms.

These individuals who were invited here to represent their companies, these individuals were not screened by FDA for their conflicts of interest, since they are representing regulated industry.

This conflict of interest statement will be available for review at the registration table. We would like to remind members that, if discussions involve any products or firms not already on the agenda, for which they have a personal or imputed financial interest, they need to exclude themselves from such involvement, and their exclusion will be noted for the record.

FDA encourages all meeting participants to advise the committee of any financial relationships that they may have with firms that could be affected by the committee

discussions.

So ends the reading of the conflict of interest statement. Before I turn the microphone over to the chair, I would like to ask, if you have a cell phone, please put it in the silent mode, so that you won't disrupt those sitting next to you. Dr. Telling, I turn the meeting over to you.

**Agenda Item: Opening Remarks.**

DR. TELLING: Thank you, Bill. I would like to also welcome everybody here today. We have a full agenda so, without further ado, I think we will get on to the committee updates. The first speaker is going to be Dr. Ferguson, who will update us on U.S. and worldwide BSE.

**Agenda Item: Committee Updates. US and Worldwide BSE.**

DR. FERGUSON: I am just going to go through. If you have my handout, you see I have a whole bunch of slides, but I am going to rip through things pretty fast.

They are pretty straightforward slides, just to update what is happening in regard to BSE around the world, and then finally, in the United States.

Just a reminder for everybody, total cases worldwide still is greater than 189,000 cases. Again, most of those, actually greater than 96 percent, have still occurred within the United Kingdom and more than 89 percent

of them occurred before 1996 and before.

So, when you still see all these numbers of cases, the vast majority of that reflects what happened in the United Kingdom in the late 1980s, early 1990s.

If you are interested in current totals, the OIE's web site actually has fairly good numbers, fairly updated numbers, about all countries that report cases.

Let's start off and talk about what has happened in the European Union. EU monitoring, since 2001, they have done very intensive surveillance, mandated by legislation.

In 2005, they have recently released their compiled report on all of the monitoring that went on in 2005, greater than 10 million tests in all the 25 member countries, in cattle.

Of those, about 1.5 million are what they call risk animals, which would be the same as our targeted population in the United States, and 8.6 million approximately are animals either 24 or 30 months old at slaughter.

Of that, 561 positive cases, 448 of those are in their risk or suspect animals, and 113 in the healthy slaughter population.

Again, in 2005, same as in 2004, both the number of cases and the overall prevalence in tested animals continues to decrease. The number of cases decreased by

about 35 percent, overall prevalence by about 29 percent.

These reductions in the number of cases, also the increasing age of positive cases -- if you read their report, there is a lot of very good information about age of cases and how that progresses over the years. I didn't include that there, because that would have made me go on for far too long this morning.

Both of those do indicate success of control measures in Europe. They also provide very good details on analysis by year of birth of positive animals.

The peak of exposure actually appears pretty well defined in a few of the member states. This is the same as it was last year, which is a good indication.

France and Ireland, that peak appears to be about 1995, Germany, Belgium, Italy, The Netherlands, the peak appears to be about 1996. So, that is a good indication that perhaps -- well, it could be one of two things.

Either that is when the control measures really started to kick in or, more important, it could also be an indication of the increased surveillance that began in 2001. It is a little bit early to tell.

They are also doing significant monitoring in small ruminants, 614,000 tests in sheep and goats. Of that, 959 positives. Obviously, most of that is scrapie. They had no confirmed BSE cases in small ruminants this year.

Just to show you what has happened over the past five years within Europe, as you see, total number of positives by year, and that is decreasing every year, the same thing here in the risk or suspect animals versus healthy slaughter.

Those are very good indication that the control measures that they have in place in Europe are working and are doing the job that they are supposed to do.

Let's move to North America and a brief update here on BSE in Canada. As a reminder, Canada has been doing active surveillance, targeted surveillance, in the population where they are most likely to find disease since 1991.

As everybody knows, in 2003, they identified their first native case, in May of 2003. After that period, they significantly increased their surveillance.

So, these are just their numbers, in 2003, about 5,700 samples, two positives. One was the one that we found here in the United States in Washington State in December of 2003. So, increasing their surveillance again, with no cases in 2004, two in 2005, and five to date in 2006.

They did provide a very detailed epidemiological summary that was made public in January of this year. They are continuing that work and hopefully will have some more updates out fairly soon.

What this summary shows, it really talks about their geographic cluster theory, and the idea that the links between rendering, feed production, livestock production, tend to occur in clusters, in a fairly well defined geographic area. It is only logical, then, that that would be where the disease could cycle, would be in that type of a cluster.

What this report shows, which went through the first five cases, I believe, it does define links between most of these cases and links in this cluster.

There are also linkages in the more recent cases, again, to that same cluster and, as I mentioned earlier, hopefully they will be putting out some more updates with as good epi work in the near future.

To focus a bit on Japan, BSE was first identified in Japan in late 2001. Actually, it was September 2001. They imposed a feed ban, than, after that first diagnosis. So, the feed ban has only been in place since 2001.

Here is the number of cases. It stays about the same here until the past two years, where it has jumped up a bit.

One note about Japan. A lot of their surveillance and the testing numbers that you see have been primarily in clinically normal animals, or animals presented at slaughter.

It has only been since about 2004 that they have really increased their focus on the targeted animals or the risk population, as we would define it.

Now, let's move to the United States. As everybody knows, we have been doing active surveillance in the United States since 1990, and we are targeting the population where the disease is most likely to be diagnosed. That is the most efficient way for us to conduct a surveillance system.

The assumption is that if we can't find disease in that population, then it is even more unlikely for us to find it in the non-targeted population.

So, we can use the data that we get from that targeted sampling to extrapolate information to the broader cattle population.

Our targeted population has always been, and continues to be, those animals that have some type of a clinical abnormality that could even remotely be considered consistent with BSE.

So, these would be non-ambulatory animals, dead stock, which are animals that die for some unexplained reason, central nervous system cases, either called to our field people or on farms.

We work with veterinary diagnostic labs, public health labs, for rabies negative animals, and then we also

work with our colleagues in FSIS, for those animals that are condemned on ante-mortem inspections when presented to slaughter.

Everybody knows we ran an enhanced BSE surveillance program that began in June 2004. Our initial intention was to run that for 12 to 18 months, with the goal of getting as many samples from the targeted population as we could.

We actually ran on a bit longer than the 18 months and ran through August of 2006, greater than 785,000 tests during that whole enhanced program. During that time frame, there were two positives identified during that effort.

Just to show on a monthly basis what we did in our enhanced program, as you can see, there is a little bit of a cycle.

With the population that we are sampling -- these are animals that are clinically abnormal in some way, and with the facilities where we were collecting, these are animal disposal facilities, rendering facilities, 3D, 4D, salvage slaughter plants.

Animals tend to get sicker, die, be culled in the winter. So, we always had a little peak in the winter. I just wanted to show folks, most folks don't quite understand exactly where we were sampling and why that

might occur.

Let me explain a bit about OIE standards. These are the international animal health standards for BSE surveillance.

This reflects changes to those standards in May 2005. This is what we are using as our guidance for how we do surveillance from here on out.

It is a weighted point system. Previously it was a simple table that said if you had cattle population X, you need to get Y number of samples.

Now it is an interesting system where it recognizes that you are more likely to find the disease in certain subpopulations.

So, you get more points for that population where you are most likely to find the disease. We have four surveillance streams.

They are clinical suspect, which would be those animals with really pretty classic clinical signs of BSE, causality slaughter -- these would be those animals -- these are European terms, sometimes they fit with North American terms, sometimes they don't.

Casualty slaughter are those animals that are clinically abnormal. They would be condemned on antemortem inspections.

So, these are those non-ambulatory animals, could

be the very weak, emaciated, thin, just some type of a subtle abnormality.

Fallen stock are dead stock, essentially, those animals that die for unknown reasons. Then, healthy slaughter is pretty self explanatory.

I don't know if you can actually read this. Hopefully you can. This shows the point system where it recognizes that you are most likely to find disease here in a clinical suspect.

So, you get 750 points for a clinical suspect between four and seven years old. That subpopulation is where you are really most likely to find disease if it is present.

You get essentially limited points for sampling in routine slaughter. So, what countries can do to use this table, you can access whatever population you like to meet the standards, and then the table says you need to get X number of points for a certain design prevalence, 300,000 points over a seven year period at a design prevalence of one in 100,000.

So, a country can then use this table and figure out what type of samples in what population they want to sample to reach those numbers of points.

So, you could sample a pretty small number of clinical suspects and reach that point value, or design

prevalence, or you can sample a much higher number of routine slaughters. It recognizes that you can access both of those populations. It is just how many samples you want to get.

We did a summary of not only our enhanced surveillance program, but also what we have done for surveillance for the past seven years. That was made public in April of this year.

Just to give you kind of a graphic example of how to do this point system, these are the points that we obtained in our surveillance for the past seven years.

So, close to three million OIE points over the past seven years in the different surveillance streams. For those who ask about this healthy slaughter one, knowing that we really are not sampling healthy slaughter animals, this is a function of our data base.

Especially in some of these earlier years, our data might be somewhat limited. If we could not pull out of the data base a specific clinical sign, to assign this to one of the other surveillance streams, by default it would go into the healthy slaughter for this calculation.

So, with that summary of data, not only did we put out there just a raw data summary, we also did an estimate of BSE prevalence in the United States.

We used two methods to do this. One is the BsurvE

model, which is a model developed by the Europeans with some input from our colleagues down under in New Zealand.

It looks at what we know from the epidemiology of the disease in Europe and factors in population data when animals are most likely to leave the population.

It can be used to help a country set up a surveillance system, can also be used to estimate prevalence.

We then also tweaked this model a bit and came up with what we call the Bayesian birth cohort model, which incorporates what we would expect to see the effects of a feed ban, which the Bsurv doesn't show, and it also sets up some linkages between birth cohorts.

We also did several sensitivity analyses in this report, just to make sure that our assumptions weren't way off base.

The overall conclusion was that the BSE prevalence in the United States is very low, less than one infected animal per one million adult cattle.

If you are interested in most likely values, with the Bsurv model the most likely value was seven infected animals, with the BBC model the most likely value was four.

With the sensitivity analyses, those values ranged from one up to about 40, which all of those then led us to this conclusion, pretty solid, that the prevalence is

less than one infected animal per one million cattle.

So, what are we doing now and where are we going from there? We have used these same methods and have moved forward into what we call ongoing surveillance.

We have been transitioning here since the end of August. What we figure is about 40,000 samples per year, again, still from this targeted population.

This will allow us to continue to monitor the status of U.S. cattle and will allow us to detect prevalence if it starts to increase.

We did this calculation, again, based on our analysis of the enhanced surveillance data and using the Bsurv model.

We first of all looked at OIE recommendations, which are at a design prevalence of one in 100,000. We wanted to make that a bit more sensitive. We wanted to stick with one per one million. So, we used the Bsurv model to estimate sample numbers and points.

Again, think of that basic premise that I described for the OIE, with a different number of points for different subpopulations.

With this, we need to get three million analytical points over a seven year time frame. When we look at what we did in enhanced, we averaged about 9.5 points per sample. So, we just divided and that gets you to

about 40,000 samples per year, assuming we will get the same average points over a seven year time frame.

That is a very quick run through of both an update of what is going on in the world, what the international standards are, and where we are headed in the future. I think I have time for questions.

DR. TELLING: Yes, you do. Thank you, Dr. Ferguson. Are there any questions?

DR. GESCHWIND: Dr. Ferguson, when you were talking about the Japanese cases, could you comment about the ages of the animals?

Before 2004, I believe they were testing every cattle within a certain age range, and that they did find BSE cattle among those that would not be identified with the current methods in the United States. Can you comment on that?

DR. FERGUSON: I am not quite sure what you expect me to comment on. I think as we all know Japan, in 2001, by their regulations, required that every animal slaughtered, regardless of age, be tested for BSE.

They have recently changed that reg slightly, and it is only animals 20 months of age and older be tested at slaughter.

They did find two animals, a 21 month old and a 23 month old -- unless my memory escapes me at this point

in time -- young animals, apparently normal at slaughter.

They were positive on the screening tests, negative on IHC, and then positive again with the way Japan has done the western blot.

They have put those into mice to see if there is transmission. To the best of my knowledge, those results aren't out there yet, but there is no indication that they have gotten any signs of disease in those mice.

Perhaps some of the researchers in this group can clarify that, if I am mistaken on that point. That is the situation in Japan.

DR. EPSTEIN: Lisa, my question is, does USDA have information about food chain controls in non-U.S. countries? Are we in a position to comment how adequate the food chain controls are from country to country?

DR. FERGUSON: I can speak for AFIS per se, since we are not the food safety group. That is really not part of the information that we have.

Our colleagues in FSIS, through their equivalency evaluations, work with certain countries, will have some information on essentially the red meat inspection and control, similar to what they would do in the United States. They would have that type of information. I am not sure how much further in the food chain you need to go beyond that.

MS. KRANITZ: Dr. Ferguson, in the United Kingdom and Japan they have found cases of BSE in animals that are not symptomatic. So, I would like to know why the USDA doesn't consider random sampling of healthy stock.

DR. FERGUSON: I think it is shown in that OIE table. We all recognize that you can pick up disease in animals before they begin to show clinical signs.

It all comes down to what is the purpose of your surveillance program and how do you best accomplish that purpose.

In the United States, the purpose of our surveillance program is animal health monitoring, to help us define either the presence or the absence of disease in the U.S. cattle population.

The purpose is not to identify each and every individual case of BSE that might be out there. In fact, that is an impossibility to do with current test methods that are there.

So, we have chosen the most efficient way by targeting that population where we are most likely to find disease if it is present, to give us sufficient information to help us define the status of the U.S. cattle population.

DR. MANUELIDIS: As a point of information, how many cows, adult cows, are there in the United States, so that the 459,000 that were tested is what percentage of the

population versus Japan.

Then the second question is, the Japanese found more cases. Would you sort of compare a little bit or say something about the method of testing and the adequacy of the American testing method in its generality as compared to the more extensive Japanese testing and European testing.

DR. FERGUSON: Let me make sure I remember it. The first question was about adult cattle populations. We estimate adult cattle populations to be about 42 million currently.

I haven't done those numbers. I am not going to stand up here and do math in my head and divide 759,000 over 42 million. I will let you guys do that, if you so choose.

As far as the adequacy of our surveillance efforts in the United States compared to other countries, we feel very comfortable and very solid with the information that we have obtained, both over our enhanced efforts for the past two years, and all of the surveillance that we have done prior to that.

The prevalence estimates that we have done uses some very solid analytical methods, we believe, to come to the conclusions that we have.

So, we feel like the surveillance that we have done, targeted in the population that we have, is

sufficient to help us define what is going on in the United States.

DR. MANUELIDIS: I am really not trying to be difficult. Perhaps you can't answer the question. I really wanted to know the specific methods that you use and how they compare to the Japanese or the Europeans.

DR. FERGUSON: Sorry, I forgot that part of the question. I assume you mean specific test methods.

DR. MANUELIDIS: Yes.

DR. FERGUSON: We are using -- actually, at this point in time, I think everybody knows we are using the Biorad test for our screening. Then, for confirmatory testing we will use both IHC and a western blot to help confirm disease.

That is essentially the same as in Europe. They have other rapid tests that are also available for use, not just Biorad, but still confirmatory testing is with IHC and/or western blot. A similar thing in Japan. They are also using Biorad, and confirmation is with IHC and western blot.

DR. HOGAN: In terms of identifying your targeted population, how are those animals being identified? Is it by government employees or by industry, and what is your sense of the compliance rate?

DR. FERGUSON: We have had very good cooperation

with the industry over the past two years, actually since 1990, since we have been doing surveillance.

As everybody knows, our surveillance is not mandatory. We do have some regulatory authority to do that, but we have chosen not to exercise it at this point in time. We have gotten where we are today with cooperation with the industry.

In our enhanced program, since our goal was to get as many of these samples as we can, it has not been an issue of picking and choosing.

It is an issue of, okay, is this animal old enough to meet our target, is it greater than 30 months of age, does it meet this target, and is the sample of sufficient quality that you can test it. If you are pouring the brain out, we don't really want that.

So, that was sort of the criteria. We have had AFIs personnel collecting samples, we have had state personnel collecting samples. We have had contractors collecting samples, where we have done the initial training, set them up on our data base, and go in and cross check on them. So, it is a wide variety of folks who are collecting the samples for us.

DR. TELLING: Okay, thank you, Dr. Ferguson. If there are no further questions, I think we should move on. The second update is from Dr. Scott, who will update us on

variant CJD epidemiology and transfusion transmission.

**Agenda Item: vCJD Epidemiology and Transfusion Transmission.**

DR. SCOTT: This is going to be a brief update of vCJD epidemiology and transfusion transmission cases.

First, I am going to mention what the total number of cases is worldwide of clinical diagnosed variant CJD.

The total number of cases right now, as of August 2006, reported on the UK web site is 196. This is both deceased and diagnosed and still alive.

These are the top three. In the United Kingdom, there are 162 cases, in France 20, Republic of Ireland four, and then there are a number of other countries that have had one to two cases each reported, including the United States.

I want to point out that, in the case of Italy and many of the cases in France -- about 19 -- and the Netherlands, those patients in those particular countries had no significant travel outside of their home countries.

So, in other words, it might be speculated that these cases were acquired endogenously. Some of the other countries had travel to the United Kingdom of less than six months, such as the patient in Portugal and the patient in Spain. The point I mean to make is that not all of these cases are directly derived from visiting the United

Kingdom.

The rate of variant CJD deaths has been declining in the United Kingdom over the past several years. This is the number of deaths from definite and probable variant CJD reported in the United Kingdom.

I am showing you from 2000 to the present, but remember that the first case was published in 1995 and probably developed symptoms in 1994.

I am beginning here with the peak year where there were 28 deaths from variant CJD in the United Kingdom. As you can see, while the years go by, you get a decline in the number of cases. So, in 2006, there were three deaths reported.

I want to point out that, in the United Kingdom right now, there are six patients right now still living with this disease. So, it doesn't look as if we are going to have a large number, as we did in the year 2000.

There have been three reports of transfusion transmission of variant CJD in recipients of non-leukoreduced red cell concentrates from donors who subsequently, post-donation, developed variant CJD. They were healthy at the time of donation.

Two of these cases had already been reported at the time of the last advisory committee meeting, but the third case was reported in February of 2006.

Two of these cases were recipients that developed clinical variant CJD. The donors to these patients developed their disease about 18 to 42 months after they donated blood.

The recipients developed symptoms of variant CJD six-and-a-half to eight years after receiving the transfusion from these donors.

The new case had a donor who developed clinical variant CJD 18 to 20 months after he or she donated, and the recipient developed variant CJD eight years after receiving that donation.

In addition there was one infected asymptomatic recipient of blood from a patient that developed variant CJD. This person died of an unrelated illness five years post-transfusion but, at autopsy, the variant CJD associated PRP protein was found in the spleen and lymph nodes of this person.

The other thing that makes this case unusual, besides being diagnosed when asymptomatic, is that they were heterozygous for methionine and valine at PRP codon 129.

So, this is a genotype of the prion protein that previously had not been reported in people with clinical variant CJD.

This was the first and the other two types are

MM, which are all the clinical cases reported so far, and VV, and we will get to that in a minute.

To continue on the same theme, I am showing you an update or new information that has recently come out concerning the study by Hilton et al that was published in 2004.

This was a United Kingdom tissue survey where anonymized tonsil and appendix samples were taken from subjects that had undergone surgery between 1996 and 1999 in the United Kingdom.

The samples that were studied were from patients aged 20 to 29. Very interesting and important, three out of the 12,674 samples that were deemed adequate for study were positive, suggesting one in 4,225 people in this age group might actually be infected with variant CJD. All of the positive samples did come from appendices.

Now, what is new about this is that prion protein genotyping was done on two of these samples. In this first sample, there was not enough to do genotype testing, but the tissue was taken and used in a transmission study into mice, and those results are pending. We don't know what has happened to those mice just yet.

In the second subject sampled, the genotype was found to be valine homozygous. So, this was the first report of an infection in a valine homozygous person. The

second was also a valine homozygous individual.

So, to summarize, variant CJD clinical cases are declining in the United Kingdom. We have had three transfusion transmission infections reported in the United Kingdom, one fairly recently.

I would just like to point out that, out of the 18 identified living recipients of blood from people who came down with variant CJD, recipients that have survived at least five years post-transfusion, now three out of 18 of these people have developed vCJD infection. Two of those are clinical and, as I showed you, one of them was preclinical or subclinical at the time of death.

This implies a fairly efficient transmission by blood. This amounts to about 17 percent. Also, we now know that all three prion protein genotypes at codon 129, the MM, the MV and the VV, are susceptible to infection.

What we don't know is whether people with this genotype ever develop clinical illness. This brings up the continued possibility that there are silent and asymptomatic infections that may never become symptomatic, but may pose a risk of iatrogenic transmission to others.

In particular, we are concerned with blood and plasma, but there are other iatrogenic transmissions to be considered as well. That is all for my update. Thank you all for your attention.

DR. TELLING: Thank you, Dr. Scott. Any questions of clarification for Dr. Scott?

DR. GESCHWIND: Dr. Scott, just regarding the Saudi Arabia case, as I recall, when that was originally presented at the academy meeting, that patient had lived in the United States for, I believe, greater than six months and, on the way to the United States had spent the night in London on the way there. Any thoughts about that, in terms of the risk of transmission?

DR. SCOTT: I think that is somewhat similar to the Japanese case, where that person spent 24 days. This has been reported so far in two different ways that I have seen.

One is the WHO report dating from June, where they state that only in France, Italy and the Netherlands did the people have no significant travel outside their home countries.

In the United Kingdom, it is reported differently, in that they looked at cumulative residence in the United Kingdom of greater than six months, how many people in the other countries had that kind of residence in the United Kingdom.

In Saudi Arabia, they report zero. So, obviously, that is a person that could have been there for a night or three months and wouldn't have been counted in the UK way

of tabulating things.

I think the question does always become, was this endogenously acquired or acquired in some other country, and it probably isn't possible to answer that for certain. It may be that a single exposure to high titer BSE could infer infection.

DR. COLVIN: In the case of the UK tissue survey, out of the patients that were surveyed, that 12,674, was it known if those people, for one thing, had had any blood transfusions or, secondly, if any of them had been users of any kind of plasma or plasma-derived products.

DR. SCOTT: That is a very good question. In order to get the study accomplished, they had to completely anonymize the samples. So, those people will never be identified and we don't have any information about them.

DR. TELLING: Any other questions for Dr. Scott? If not, thank you very much. Our next speaker is Dr. Williams. He is going to talk about a draft guidance for industry, an amendment. He is going to be talking about a donor deferral for transmission in France since 1980.

**Agenda Item: Draft Guidance for Industry:  
Amendment.**

DR. WILLIAMS: Good morning. I am going to present a very brief update on recent draft guidance issued by FDA pertaining to deferral of donors with a history of

transfusion in France since 1980.

The current recommendations are for vCJD related donor referrals, as have been seen by this committee many times, but I wanted to run through them quickly just for the new members of the committee.

Most of these, or all of these, are captured in guidance to industry published in January 2002. Deferrals include residents with travel of greater than or equal to three months in the United Kingdom from 1980 to 1996, residents with travel of five years or greater in Europe for the same period of time.

For donors of source plasma, this criteria applies only to France, which is considered to have five to 10 percent consumption of UK Beef, and therefore be at proportionately higher risk compared with the rest of Europe.

Combined with the presumed prion production reduction in the course of fractionation, this deferral was modified specific for plasma donors.

In addition, donors who spent greater than or equal to six months on U.S. military bases in Europe between 1980 or 1990, or 1980 to 1996 respectively for regions in the north and the south, are deferred.

This is based on importation of UK beef into U.S. commissaries, and this differed between the northern and

southern bases during this period of time.

The guidance also defers donors for history of transfusion in the United Kingdom from 1980 to the present and for receipt of bovine insulin sourced in the United Kingdom after 1980.

At the meeting of this committee on October 14, 2004, the committee reviewed current FDA regulations regarding vCJD related donor eligibility.

After considerable discussion, they did not make recommendations for further FDA actions to protect the blood supply.

However, there were discussions at the meeting concerning the predictive value of donor questions that were used to exclude TSE risk and just how effective the questions were, as well as the feasibility of deferral for history of transfusion outside of the United Kingdom, but no specific recommendations were made at that meeting.

Subsequently, at the February 8, 2005 meeting of the committee, FDA brought the issue back for consideration, based largely on several recent observations at that time.

At that time there were two observed variant CJD transmissions associated with transfusion and it was recognized that two recent variant CJD cases observed in France had had a relatively large number of prior blood

donations.

That is not necessarily a scientific rationale for considering the issue, but it really did raise the visibility of the fact that any potential patient could be a blood donor.

At the time, as well, there were some actions in Europe where donors were deferred for any previous transfusion.

In France, this had been in place for some time since 1998, in The Netherlands deferral was implemented for any history of transfusion in 2004, and in the United Kingdom this took place in 2005.

Also discussed at that meeting was the potential impact of any increase of donor deferral for history of transfusion.

The history of transfusion deferral for the United Kingdom had already been accomplished. The calculations for this are reflected in the transcript, but were estimated to be about two per 10,000 donors.

Computed proportionally, the estimated loss for donor who had a history of transfusion in France was figured to be 1.4 per 10,000 donors.

Also, under discussion at that meeting, was the potential for deferring any donor who had a transfusion anywhere in Europe.

When considering this excluding the United Kingdom, that would add another three per 10,000 donors. There were no data available regarding source plasma donors and their history of transfusion or travel, but simply due to the younger age group of the source plasma donor, this would be expected to be somewhat less.

In the TSEAC deliberations at that February 8 meeting, TSEAC recommended deferral of blood donors transfused in France since 1980 by a vote of 12 for, three against, and one abstention.

However, the committee did not recommend deferral of blood donors transfused elsewhere in Europe since 1980 by a vote of zero to 15 against and one abstention.

By a somewhat mixed vote, the committee also did not recommend deferral of plasma donors transfused in France. That vote was five for, seven against and seven abstention, or other European countries, with a unanimous vote of 16 against.

In issuing its draft guidance for industry, FDA is basing the guidance on the rationale of being prudent preventive measures to help prevent or reduce the risk of vCJD transmission by transfusion.

These factors are the relative likelihood of dietary BSE exposure in France - and this has been an underpinning of any of the discussions that have been held

through the years about potential donor exposure.

At the time of assembling the guidance, there had been three presumptive cases of variant CJD transmission by transfusion. So, this was no longer a theoretical possibility.

There were 14 definite or probable vCJD cases observed in France. It is now observed that the variant CJD incubation period may be as long or longer than 12 years, and asymptomatic prionemia may be over three years prior to the expression of illness in an infected donor.

With respect to plasma donations, experimental studies of prion reduction and fractionated plasma are reassuring.

However, not all fractionated products have been studied and observations do not necessarily reflect the blood form of the variant CJD agent.

So, the guidance itself is published as a draft amendment to the January 2002 guidance entitled, Donor Deferral for Transfusion in France since 1980.

FDA recommends deferral of donors who have received a transfusion of blood or blood components in France since 1980.

This applies to whole blood and blood components intended for transfusion, as well as blood components intended for further manufacturing into injectable products

including recovered plasma, source leukocytes and source plasma.

The implementation target for this is within six months of publication of the final guidance and the mechanism that will be used.

This amendment is published as draft, but the 2002 guidance will be updated and published in final to incorporate this as final guidance. Thank you.

DR. TELLING: Thank you, Dr. Williams. Question?

DR. HAMILTON: Has there been any consideration in the Untied States of the areas of the country that the people tend to eat brain matter and offal, consuming that? Has there been any consideration of that?

DR. WILLIAMS: There has been consideration of it. In fact, the NHLBI sponsored red study actually did a survey of consumption of brain matter and had some preliminary data on that.

I think this has been an area of consideration but I think to date the scientific evidence supporting this as a potential factor in transmission of disease hasn't been strong enough to support this as a deferral.

DR. TELLING: Thank you very much. If there are no further questions we can move on. Dr. Cai from Telecris Biotherapeutics, will discuss some critical factors influencing prion decontamination using sodium hydroxide.

This is a PTTA collaborative study.

**Agenda Item: Critical Factors Influencing Prion Decontamination Using Sodium Hydroxide.**

DR. CAI: Good morning. Thank you very much for inviting me. On behalf of the Plasma Therapeutics Protein Association, I would like to present the results of a collaborative study designed to understand the critical factors influencing prion decontamination using sodium hydroxide.

This work was primarily done at Telecris Therapeutics. It used to be Bayer. Some of the work was done at Bioreliance.

So, we are all working together to establish a systematic, comprehensive approach to minimize the risks associated with potential SE transmission.

So, as the first line of defense, we have donor deferral and material control. As a second line of defense we have the capacity of the manufacturing processes to remove or clear prions.

In supplement to that capacity, we have cleaning and sanitization procedures. If those procedures can inactivate prions, that would add additional benefit in terms of risk reduction. So, this is my focus of the talk today.

So, speaking of prion inactivation, we know that a prion is very resistant, very difficult to inactivate,

using conventional viral inactivation methods, simply because, on the one hand, it is a nuclear acid base to viruses and we are dealing with protein prion infectious materials.

However, the prion has its own vulnerability, and it has its own weaknesses, because proteins can be unfolded under many circumstances.

Over the time, researchers have developed many methods to inactivate prions, including enzymatic digestions coupled with detergent treatment, and also acid or strong base treatment, or also some other chemical reactions such as titanium dioxide, photocatalytic inactivation.

Let's not forget our old friend, which is the strong base, which has been used widely in the industry to clean equipment, which includes potassium hydroxide and sodium hydroxide.

Those are readily available and inexpensive, rapid, ineffective and comparable with most of the major equipment, like stainless steel equipment, although it is not very comparable with silicon based materials. Those can be treated or disposable.

So, actually, over the time, researchers performance many studies on sodium hydroxide in terms of inactivation of prions, all the way back to the 1980s.

They used various spiking material or model prion agents for either rodents or humans, and they examined various concentrations, various temperatures, as well as various incubation times in terms of treatments. Of course, you got various results as well.

Then this is a reduction factor of the output prion titer relative to the input after specific treatments. You can see there are various reduction factors based on the conditions.

However, if you look closely, whenever there is a presence of detergents, there is a good reduction. So, please keep this in mind, and this is very relevant to our discussion.

Secondary, you can also observe that among these reduction factors, you know, most of them are quite effective.

However, not all of them give a complete inactivation. In other words, there is still residual infectivity or prions remaining after the treatments. So, the question is why.

That is quite consistent to what we have observed during the early stage of our study, the Bioreliance. In this case used scrapie brain homogenate, which contains hamster prions at one percent, and mixed with sodium hydroxide at .1 molar, incubated at 18 degrees.

The top panel is the titration of the input material. So, you can see quite significant amounts of prions gives us -- this is a half log dilution.

After the treatment you can see the majority of the signals -- the signal strength is significantly reduced. However, there is still remaining signal.

So, there are two questions here. One is, what is behind this dramatic reduction after the treatment. The second question is, what is the nature of this residual signal.

So, we set to address these questions by designing experiments that would mix the purified scrapie brain homogenate with sodium hydroxide, and incubate it with or without two percent sarkosyl, which is a detergent, after incubation at the various temperatures and the various time periods.

Then the sample is withdrawn and neutralized and treated with proteinase K in order to detect the pathogenesis conformation, as is run by electrophoresis and detected by western blot to see the signal strength of the prions.

Now, what we observed was that if you have detergents in the sample, then the residual signals can be eliminated to below the detection limit of western blot.

On the upper left panel at four degrees, you have

this much of input material. In the absence of sarkosyl, after 60 minutes, we observed about three logs of reduction with a residual signal.

When the temperature is elevated, then the reduction is slightly increased. However, in the presence of sarkosyl, at 15 minutes, the reduction is increased and, at 60 minutes, the reduction is more than 4.6 logs. So, it reduced below the detection limit. At the elevated temperature, this disappearance occurs earlier.

So, we know that a detergent mainly affects the lipid composition or the disrupted structure of the lipids and detergent has very little effect on the overall structure of proteins.

So, it is highly possible that, in the sample, there are two subpopulations of prions. One is protective by lipid components and the other is protein alone.

So, this one is protective against, not accessible, by the sodium hydroxide. So, after the treatments and when you use proteinase K to probe the structure, obviously this structure is not going to be digested, resulting in a remaining signal.

Now, in the presence of detergents, the protection is removed and the entire population is vulnerable to sodium hydroxide or extreme pH. Then, when you use this probe to probe the structure, then the

structure is no longer there.

Now, prions are about protein folding and miscoding. So, it can exist in a normal conformation with alpha helixes and exposed epitopes. Those epitopes can be accessed by antibodies as well as proteinases.

It can be mis-folded into this pathogenic form. So, in this case, the structure is mainly beta sheet and the epitopes, some of the epitopes, are buried and no longer accessible by the antibody or proteinases.

Then in order to inactivate this moiety we need to somehow unfold it or degrade it. So, this is the place, I think, that is for the prion activation.

So, this experiment was designed to further analyze the conformational change behind the prion inactivation or the structural change of prion proteins upon the incubation with sodium hydroxide.

In panel A, which is in the absence of detergent, and absence of proteinase K treatment, the trace buffer saline is a control.

It gives an input, the titer of input sample, and sodium hydroxide treatment you can see pretty much remaining, the signals are pretty much remaining the same, with very little reduction.

That tells us the peptide chain backbone is pretty much preserved after the treatment. However, the

conformation is no longer there because in the panel B, once you use proteinase K to probe this structure, the structure is obviously greatly damaged, with a big reduction in terms of titer. However, there is a little residual signal again.

Now, in the presence of detergents, again, the protection was supposed to be removed after sodium hydroxide treatment.

There is 90 percent of the signal that was reduced in the absence of proteinase K, which tells us actually the peptide chain in this case, or the side chains of the epitope are damaged.

In the presence of both detergents and proteinase K we can see the signal disappeared completely using this assay because the structure is unfolded and it was digested by proteinase.

To further support these observations we conducted immunoprecipitation assays, tried to demonstrate that the sodium hydroxide unfolds the prions.

So, in the normal conformation this epitope is exposed to the solvent, accessible to antibody. If you have antibody beads, then you can immunoprecipitate the structure.

That is what we observed for this amount of input material in the buffer control, or preneutralized sodium

hydroxide, which says that there is the same amount of solutes of the base, but it was neutralized before.

So, the sample does not experience extreme pH. So, in both cases you can see a good recovery of the signal by immunoprecipitation.

Now, it is totally opposite to the pathogenic conformation, where the epitopes are buried and you will not be able to precipitate it. In this case you see no or very low signal.

Now, treatment with sodium hydroxide, it unfolded this structure and made this epitope accessible. So, you can see the immunoprecipitation.

This is pretty much, the overall consequence, is pretty much similar to what guanidine kinase does, which is chemotropic agent, unfolds the prion, which is used as a control in this case.

So, in summary, we think the critical effects influencing decontamination using sodium hydroxides include, of course, the concentration of the agents and the presence of detergents, and temperature and time also contributes, to some extent. So, overall, the sodium hydroxide works by unfolding and degrading the structure of the prion.

So, I would like to thank people who contributed to this study, especially the PPTA collaborators. Also, the

experimental work was done by Dr. Pat Bauman and her research team.

I would also like to thank contributors, former PPTA members, as well as others who contributed to this study. Thanks.

DR. TELLING: Thanks, Dr. Cai. Are there any questions?

DR. SALMAN: Can you comment on what type of media you used for the prion? What type of vehicle do you have it in?

DR. CAI: You mean the spiking material?

DR. SALMAN: Yes.

DR. CAI: That was clarified brain homogenate from masters with the 263K strain.

DR. SALMAN: Have you tried to see if there is any type of effect of the organic matter on the decontamination?

DR. CAI: We haven't specifically examined it in that respect.

DR. SALMAN: I have another question. What is the reason to decide, as far as the maximum temperature of 18 centigrade?

DR. CAI: We chose several temperatures, such as four degrees, 18 degrees. Actually, those are conservative. So, we tried to model production processes,

manufacturing processes.

During manufacturing, the cleaning procedures vary depending on manufacturer, such as whether it is upstream, is it downstream, whether it is -- the condition of the equipment.

So, there are hundreds of standard operation procedures for each manufacturer to define the specific procedures for cleaning.

All those procedures are validated based on a validation package, you know, according to common practice, where you use many measurements to determine how much residual protein is remaining.

Often we use total carbon measurements. So, if the total carbon measure is under a certain level, then you are confident there is no residual, or small amounts of residuals, remaining.

In that regard, back to your question, it is an organic compound and those effects are probably very limited.

DR. MANUELIDIS: I would like to sort of clarify something here, to make sure that in the rest of the meeting this is clarified by the speakers.

The question is, did you ever inoculate any of this material to see how infectious it was? You are making an assumption about inactivation of abnormal prion protein

and infectivity, which other types of studies -- there are many numerous studies including heat inactivation, guanidinium, et cetera, where the correlation is not there.

So, I would really like to know, did you do any infectivity studies? I think it can be misleading to sort of say that we have inactivated this as a titer. Titer usually refers to infectious titer as measured biologically.

DR. CAI: We did do infectivity study, although the data is not shown here. We observed quite good correlation between the inactivation measured by western blot as well as hamster bioassay.

In addition, previous studies also demonstrated, as listed here -- a lot of them were done by using a bioassay, for example, infectivity as it was labeled as a green star here. Many of those cases are done by bioassay. So, they have quite good correlation.

DR. SEJVAR: Just to clarify, the conditions that you have been describing would be compatible with real life experience, in other words, actual decontamination of, say, surgical instruments, et cetera.

DR. CAI: Actually, there is some subtle difference between surgical instruments and the manufacturing processes for plasma products.

A surgical instrument is in direct contact with

central neural systems and often has a much, much higher degree of protein binding to those instruments.

The manufacturing equipment, on the opposite side, has -- it is rigorously cleaned, of course, but if you think about if there is any donation got into the manufacturing side, the infectious titers could be very, very low.

So, it is kind of a different scenario but the general approach should be applicable to both. A lot of the surgical instruments are also treated with sodium hydroxide.

DR. SEJVAR: But you are talking about specifically plasma products, et cetera?

DR. CAI: Right.

DR. MASTRIANNI: In your experiments you used sarkosyl at two percent. I am wondering if you did a titration curve to see if there was a dose response that correlated with increasing levels of detergent to show a decrease in signal of western blot.

DR. CAI: That is a good point. Although we didn't titrate it, we did a spot test. You do need a certain amount, one percent, two percent, but if you go down to .1 percent, the effect will be reduced.

DR. COLVIN: As opposed to the indirect method of looking at the structure of the prion protein through

either proteinase K susceptibility or through the western blot through antibody affinity, did you look at any direct measures of conformational change, such as using circular dichroism, NMR, spectroscopy, something that would show there has been a change, or even differential centrifugation of the products?

DR. CAI: Limited by our methods, we were unable to use a lot of physical means. We did these studies primarily in the BSL-2 lab in our setting, which is set up for pathogen research. So, we don't have extensive physical characterization testing available.

DR. GESCHWIND: Related to those last two questions, one is, there is clearly a difference in inactivating human CJD as in animal, as shown in the Peretz paper, Journal of Virology that came out recently, where human prions were found to be 100,000 times more difficult to inactivate than in animals, hamster 263 prions.

I think that is an important point to consider, that the only effective way of testing really should be -- I think this is an important point for the committee to consider for the next two days, is that really the human prions are going to be different than animal prions and we have to realize that.

Then the second issue is, is there any equipment in the processing for the plasma that would be exposed to

metal, as clearly there is a difference between brain homogenate and testing in which they have looked at small pieces of metal put into the brain.

So, brain homogenate has always been easier to inactivate than the steel rod method. I am just wondering if there is any possible exposure to metal in particular during any of the processing.

DR. CAI: Yes, the first question about Dr. David Peretz' paper, I guess using acid in combination with detergent to inactivate prions. So, they compared between hamster and human materials and there was a big difference.

I would like to point out in that case it is weak acid at a pH 3, 4. In our case, sodium hydroxide or potassium hydroxide is a strong base. It is a strong electrolyte. So, they are very, very different in their nature.

In addition, the previous studies demonstrated by almost 10 groups using the sodium hydroxide treatments with the absence of detergents, they consistently demonstrated significant removal or reduction using various strains of material, including rodents and humans.

So, the second question is whether the manufacturing process has metal components in the equipment. The answer is yes. Of course we use a lot of metal equipment, including stainless steel, all of that.

Several groups, including Dr. Safar's group, they use metal instruments into rodents to detect the prion infection. That is a very good approach and gives us a better understanding.

The study we did, you know, they were designed to address in general those conformational changes, and to understand the significance of a residual signal, how to remove residual signal. Those studies should be complementary, I think.

DR. TELLING: Thank you. We need to move on. The final speaker in this update section is Dr. Safar from the University of California, San Francisco, who will be talking about human prions clearance in plasma lipoproteins.

**Agenda Item: Human Prions: Clearance and Plasma Lipoproteins.**

DR. SAFAR: First of all, I would like to thank the committee for this opportunity to present some new data that we think are very relevant to the task of this committee.

I think that fundamental issues facing the prion research, I have selected those that I think are very relevant for this meeting and for the present agenda.

I think that the three in the square are interrelated. The pathogenesis of the prion diseases is

important to understand and to plan the most effective therapeutics which would halt the prion formation and remove existing prions.

The condition for that is to have a very sensitive presymptomatic diagnostic test. If we would initiate any therapy late in the symptomatic stage of disease, there is very little hope for the recovery. The situation is very similar to Alzheimer's disease.

This is a table which I put in. It is very complex, but I think that it is really important to realize the progress of the field in the last few years.

There were originally described two entities related to the prion diseases, normal PRP, cellular form of the PRP protein and the resistant form of PRP, PRP<sup>SC</sup>, which is infectious.

We found that there are very similar species, which actually in many cases is dominating disease, we called protease sensitive form of PRP<sup>SC</sup>.

They have different conformations. The PRPC has exposed most of the epitopes against monoclonal antibodies, where PRP<sup>SC</sup>, both S and R forms, those epitopes -19 and -125 are already buried.

The secondary structure of PRPCs is 40 percent helix. RPRP<sup>SC</sup> is 40 percent beta helix. We don't know the conformation of the PRP<sup>SC</sup>.

Quaternary structure of PRP<sup>SC</sup> is a monomer PRP<sup>SC</sup> or oligomers, and SPRP<sup>SC</sup> can polymerize into analoid-like rust.

Standard PK destroys both PRPC and SPRP<sup>SC</sup>, but leaves behind a proteolytic fragment of PRP<sup>SC</sup> which we call PRP22-30 by molecular weight.

Current PK, which we found very simply, hydrolyzes selectively PRPC and leaves behind a proteolytic fragment which is typical for SPRP<sup>SC</sup>. So, that is the first really direct evidence that this comes separately from what the RPRP<sup>SC</sup> comes from.

Another way to separate PRPC and PRP<sup>SC</sup>, both S and R, are polyoxometalate, polyoxometalate precipitation. Non-denaturing detergents are solubilizing PRPC, they have mixed effect on the SPRP<sup>SC</sup>, they don't solubilize RPRP<sup>SC</sup>.

Infectivity, normal protein is non-infectious, of course, and the RPRP<sup>SC</sup> is infectious. Levels during infection, there is no change, no up-regulation of PRPC. SPRP<sup>SC</sup> and RPRP<sup>SC</sup> are in equilibrium, which is typical for different prion strains.

In RML infected mouse, the clearance half time for the SPRP<sup>SC</sup> and RPRP<sup>SC</sup> is 1.5 days. I showed this slide because I think it is becoming increasingly important.

There is a large percentage of the sheep scrapie which carries selectively the SPRP<sup>SC</sup> forms, and wouldn't be detected without the availability to detect this PRP<sup>SC</sup>. We

saw it in Norway cases and more and more cases in Europe.

There is a growing number of human CJD cases, and they were presented last week in San Francisco by Luigi Gambatti(?) from his CJD surveillance collection.

He estimates that it may be up to 14 or 15 persons which display selectively SPRP<sup>SC</sup> and practically no detected RPRP<sup>SC</sup> proteins.

So, this is becoming very important for two reasons. First, practical detection and identification of the prion disease. Second, in a theoretical sense, how is it related to the disease and how important is it in pathogenesis.

The direct PRP protein with proteases, we designed 10 years ago the protocol which avoids proteinase K. It is called conformation dependent immunoassay, and it recognizes antibodies which are exposed in PRPC and hidden in PRP<sup>SC</sup>. This is the beta helix of PRP<sup>SC</sup>, helix A and C, which are still remaining there.

If you test simultaneously one sample which is native and the second of which is denatured after denaturation with sodium hydrochloride, you compare the signal.

If you don't see any increase in the signal, you know that they are PRCP proteins, or if there is a very small increase, you can account for it by establishing for

the size of cattle.

If there is an increase in the signal after denaturation, you know that you have a certain percentage of PRP<sup>SC</sup> in the original sample, which had hidden epitopes. that is a quantitative parameter indicating the presence of PRP<sup>SC</sup> protein.

We weren't very happy with the sensitivity. So, we are looking for the compounds which would selectively precipitate PRP<sup>SC</sup> and leave behind PRPC. One of those compounds was the keggin structure of polyoxometalate, where the phosphate is in, and they are coordinating the constant oxide of the hyderons around.

There is a misconception that it is some small cell. The polyoxometalates are actually very large. The monomer of PRP structure, monomer of PRPC is about 1.8 nanometers. The polyoxometalate in this case, kaggin structure, is about one nanometer large.

So, those are very large compounds which can be synthesized in a way which modulates either size, shape or charge.

We found out that some of the polyoxometalates are efficient in aggregating PRP<sup>SC</sup>, the S and R forms, and large polyoxometalates actually have a positive effect. They dissociate PRP<sup>SC</sup> proteins.

This dual effect is still not understood exactly

at the molecular basis. It is definitely related to the charge and size polyoxometalate.

So, kaggin structure, small polyoxometalate cyclates facilitate prion formation and decrease cell growth from prions, and the large polyoxometalate have the opposite effect. They dissociate PRP C protein and they make smaller complexes.

Our studies of SVRPLC proteins, the protease sensitive form, was initiated with generating biogenic systems to regulate the PRPC level of expression.

So, we could shut down PRPC and look what happens to PRPC proteins, both the S and the PRP<sup>SC</sup>. The first surprise came following the incubation time.

When you shut down PRPC expression and then follow the incubation time of the animals, those which express downregulated PRPC from 100 percent to about five percent of residual expression, extended their incubation time by about three-fold.

So, that was a really amazing result. We didn't expect it because we were afraid that the small leak we have in the background would inevitably lead to very small changes in incubation time.

When we measure the PRPC protein prions, we found that the PRPC has a half time of about 18 hours, PRP<sup>SC</sup> has a half time of about 36 hours, measured by both CBI and

western blots.

So, that was really an amazing finding, indicating that this is a very powerful mechanism in the brain, physiological mechanism, which is able to clear prions in one and a half day, and practically 50 percent of already formed prions.

It is apparently related also to the strain. When we compare CO1V, an animal strain, CO1V, which has a slightly extended incubation time, it cleared about twice as more slowly. Also, the accumulation was slower.

So, there is an interrelationship between the stability of the prions for prion strains, incubation time, the accumulation rate and clearance rate. Those functions are strain specific.

If you look at the pathology of the animals where the PRPC was likely to be expressed during the incubation time of prions, we see large deposits of PRP<sup>SC</sup> proteins.

If you look at animals which were inoculated and then, after 98 days, which is about two thirds, we shut down PRPC, we see how clean those brains are.

There are only some deposits in the corpus callosum in the white matter. Most of the cellular areas are completely clean. Other deposits are around the vessels.

This slide, I think, is really optimistic. It has

got a therapeutic approach. If we would be able to down-regulate PRPC, you would effectively cure the disease because the brain has a very powerful clearance mechanism for clearance.

Additionally, the therapeutic level, we know now that it is possible that the prions are continuously synthesized at the low level and that the brain has -- that they have some physiological function in the brain, and that the brain has, at the same time, a very strong clearance mechanism, which is how you get rid of them.

So, where do prions go? We know from other experiments, when you inoculate directly prions into brain, 99 percent are lost within the first 24 hours.

So, there is massive outflux of prions from the brain, and obviously the target in this case has got to be the first circulating blot and cerebral spinal fluid.

There is no question that there is infectivity in the blood, and there are many studies indicating them. The issue in this case is which compartment of blood it is. Is it out of blood or plasma or both.

So, we established a system where we looked really blindly in both plasma and white blood cell compartments. White blood cells are sorted by facs, flow cell activated cell sorting, and by myelin Bs, and we have tested up to now granulocytes, monocytes, CD4, T cells, B

cells, circulating dendritic cells from different animals and also from CJD patients.

The results are -- the results really increased in plasma. We started to supply them with polyoxometalates. Polyoxometalates have not only very specific precipitation capability of prions, but they also precipitate lipoproteins. They have a still not fully understood affinity to the lipoproteins.

So, we decided to test where the prions would go in the human plasma by using polyoxometalate fractionation. By increasing concentration of polyoxometalates, you can supply plasma into the LDL particles, lipoprotein particles, immunoglobulins, HDL and other plasma components.

When we spike the plasma with prions from brain, sporadic CJD prions -- so, this is a homologous system, plasma, human plasma, and sporadic CJD and one case of CJD.

We found that all the prions, by western blots, and by CDI, were fractionated or precipitated into the VLDL or LDL fraction of the lipoproteins.

It is not actually so surprising. The PRP, prion PRP has a very high affinity for cholesterol. It is very difficult to separate them.

So, the lipoprotein particles that are about 60 percent of cholesterol and phospholipids, and about 30

percent hydrophilic proteins. So, the fact that they have affinity for each other is not very surprising.

What came up as a surprise was the level of the affinity. When we tested in our affinity assay, the binding of the sporadic CJD prions to the lipoproteins coated on the plate, the mid-points which was the indication of the affinity constants, they were in the low picomolar range, between 30 to 100 picomoles.

That was a real surprise. The second surprise was the selectivity. When we coated the plate with HDL, there was practically no specific binding, no cooperative binding.

So, despite a similarity in the lipid content between LDL and HDL, there was a big difference in the affinity for the LDL, a preferential binding for the LDL.

The second surprise came from electron microscopy when we purified the sCJD protein from the brain and then incubated them with the VLDL and LDL, or HDL.

We saw decoration of the human neurons only with LDL. We didn't see any decoration with HDL. If you compare the signal of our best monoclonal antibodies, and decoration with LDL gold, we see how few dots we actually got on those prion neurons. On the other hand, we have a massive accumulation of the LDL on the human prions.

The common component to all the VLDL, LDL, IDL,

and not HDL, is the apoprotein B. The other apo-C, E and so on, are exchangeable, but they are not present in LDL.

So, we decided to test specifically the apoprotein B, purify for protein B. The affinity was only about four to 10-fold lower than the affinity of the original LDL.

So, in conclusion, the PRP SC protein, both the S and R forms, have a very high affinity for lipoproteins containing apoprotein B, or apoprotein B itself.

The binding is conformationally specific. If you compare the affinity constants of the alpha helical PRP versus random coil PRP, versus native PRP SC protein in prion neurons, they go in that sequence.

So, alpha helical PRP doesn't have practically any binding, random coil higher, and then followed by the native prions.

The stoichiometry is also is also different between recombinant PRP and the native prions, where we see the binding ratio about three, we see only one to one ratio for recombinant PRP in the random coil conformation.

The LDL suffers from a misconception. Most of the people have the impression that LDL is cholesterol. That is actually not true.

It is about 30 percent protein, which is called apoprotein B. It has a molecular weight about 550

kilodaltons. It has 4,536 amino acids.

It composes about 30 percent of the weight of the LDL particle. The rest is cholesterol and phospholipids in the monolayer, and the apoprotein B, which is hydrophobic alpha beta sheets and alpha helices, are basically wrapped around the particle and presents no specific fusion with the cell.

If there wouldn't be apoprotein B, we would die from atherosclerosis of age two or three, probably. So, it is a very important mechanism which, through the LDL receptor domain, directs the LDL particles to the cells, which express the LDL receptor and, if they meet, influence of the cholesterol. So, it is a very important regulatory mechanism.

Is it conformation specific also from the other side? In the prions, when we test the different prions from CJD, sporadic CJD, CN hamster(?), scrapie, RML, we saw a totally different binding curve, indicating different stoichiometries and different affinity constants.

So, it is not only conformation specific for PRP, for human PRP, but it is also able somehow to discriminate between PRP, different prion strains.

So, human LDL and apo-B binding with AP CJD prions, it is conformation specific. It has a very low affinity constant down to 30 picomolars.

The PRP affinity for binding is present in 19 to 31. The order of the binding goes from the beta HPRP to the denatured PRP to the alpha helical PRP.

The different stoichiometries, three to one for native prions, one to one for recombinant PRP. The lipids of LDL are not essential for the binding. Glycolipids and glycosylation are not essential for the binding in PRP. LDL and APO-B binding to denatured PRP is sequence specific.

So, did we look into sporadic CJD cases. I think that the first step before that, we actually realized that first we have to validate our assay.

We have to show that we have a -- that we can truly detect PRP<sup>SC</sup> protein and, second, that we can truly measure quantitatively PRP<sup>SC</sup> protein, and correlate it with the prion infected.

So, in this study, which was actually initiated with Glenn Telling, and whose transgenics he generated in San Francisco, we inoculated three different cases of sporadic CJD in the end point titration experiment in different transgenics to determine end point titers.

At the same time we made homogenase from the brain and tested by CDI, the dilution curve, in parallel. When you see the correlation, there is a very clear overlap.

It shows one important difference. The 50 percent transmission rate indicating one infectious unit per ml, at that level, CDI has a reading skill of about 20,000, which is about 1,000-fold over the capability of the CDI assay. So, in effect, the CDI is more sensitive than the bioassay in transgenics.

So, one more question was correlating the established procedures of immunohistochemistry and pathology with the infectivity and with the CDI.

So, we blindly tested PRP C protein in those different forms of prion diseases and in 18 different anatomical areas in eight sporadic CJD cases.

We could detect the RPRP<sup>SC</sup> protein everywhere. In contrast, the immunohistochemistry and localization profiles in many areas the sensitivity of both was not exceeding 20 percent, or was even lower than that.

So, one conclusion. First, the testing has to be in a diagnostic aspect. It has to be in different anatomical areas.

Second, the CJCDI shows absolute diagnostic sensitivity and specificity in all of those anatomical areas.

The second important finding was related to the SPRPSE. When we looked at the concentrations of RPRP<sup>SC</sup> versus SPRPSE, in all frontal and white matter areas we

tested, there was more SPRPSE protein over the RPRP<sup>SC</sup> protein. The RPRP<sup>SC</sup> protein actually formed about five to 10 percent of the total.

The next presentation is going to be humoral. This is just the first data showing the CDI on the VLDL fraction and plasma of the CJD cases.

When we tested total PRP concentrations in 21 donors and 20 sporadic CJD cases, we didn't find any difference in total PRP or in the SPRP and the PRP<sup>SC</sup> protein. It is below the threshold.

When we separated VLDL, there was a significant difference in the sense of more total PRP protein in the VLDL from sporadic CJD cases, and most of the total PRP increase was RPRP<sup>SC</sup> protein, actually.

So, what are the conclusions? I think the apoB containing lipoproteins are strong candidates for carriers of sporadic CJD prions in human plasma, and I will talk about it as the diagnostic implications emerge.

Binding of apoB containing lipoproteins to native sporadic CJD prions is conformationally specific with Kilodaltons down to 30 picomolars.

The existence of highly stable lipoproteins prion complex in plasma suggests that it may be that the lipoproteins have a role in the clearance of prions from the brain and other tissues.

Both conformational specificity and high affinity will lead to the development of new assays for prions.

The data on low affinity prion ligands suggest that lipoprotein binding may impact the infectivity of sporadic CJD prions.

Conformational specificity of the apoB binding may lead to the new ways of differentiating human prion strains, including variant CJD.

Plasma lipoproteins provide, in my opinion, highly specific ligands for prion removal from plasma. Thank you.

DR. TELLING: Thanks, Dr. Safar. We have time for maybe two quick questions before the break, if there are any?

DR. MASTRIANNI: I have got so many, but I can talk to you later. One just generalized question. Why does LDL bind scrapie better than PRPC? Do you have a conformational model for that? Maybe I missed it.

DR. SAFAR: We don't have a really good conformation model of apoB. ApoB is almost as difficult to study structurally as PRP<sup>SC</sup>. It is very large, very hydrophobic, and there are no three-D structures.

The model I showed is a computerized approximation of the CD infrared spectroscopy of the entire EM. So, it is a very large protein.

On the other hand, there are many tools in molecular biology, including fragments of apoB and LDL, which will allow us to determine which domains specifically, in the first approximation, are responsible for the binding and we are studying it now.

DR. MANUELIDIS: If I understand it, you are saying that if you take out the LDL fraction of plasma, you would lose a lot of the infectivity in prions. Is that correct, and is that true?

DR. SAFAR: We think that practically all the PRP<sup>SC</sup> from the CJD brains spiked into the plasma co-precipitated with LDL and VLDL, yes. We haven't measured the infectivity.

So, to your question, we measured PRP<sup>SC</sup> protein, which we correlate with the CDI, which we correlate with viruses.

So, we are very sure that what we detected is infectious PRP<sup>SC</sup> protein. Formally, we haven't done a bioassay yet.

DR. TELLING: Thanks, Dr. Safar. I would like to keep this on track, which we more or less are. I would like to take a break and reconvene at 10:30.

[Brief recess.]

**Agenda Item: Topic One: Experimental Clearance of TSE in Plasma-Derived FVIII Products.**

DR. TELLING: We are going to move on to topic one, experimental clearances of transmissible spongiform encephalopathy infectivity in plasma derived factor VIII products.

We are going to start out with subtopic A, BSE clearance studies of factor VIII, study methods and clearance levels, and a presentation by Dr. Scott from the FDA.

**Agenda Item: TSE Clearance studies for pdFVIII.**

DR. SCOTT: Mine is the first of two presentations. The second will be given by Dr. Thomas Kreil, representing the Plasma Protein Therapeutics Association.

We are going to have somewhat different presentations. What I would like to do is outline some of the challenges in TSE clearance studies, and to introduce the committee to some discussion questions that we would very much like your scientific input on.

Our TSE safety concerns are that, theoretically, plasma derivatives might transmit variant CJD or other TSE agents, since we certainly know that blood can do this and that plasma of infected animals is also infectious.

Any such risk is probably very low, based on the fact that no cases of variant CJD have been reported worldwide in any recipients of plasma derivative, including

in the United Kingdom where vCJD risk is greatest.

However, we seek to assure the safety of plasma derivatives, especially plasma derived factor VIII, against the risk of transmission of TSEs.

The clearance of TSE agents in manufacturing of plasma derived factor VIII and other plasma derivatives has a major impact on estimated risk. In a minute I will go through what I mean by clearance and clearance studies.

In a risk assessment, a draft risk assessment that we published on the internet after the TSE advisory committee meeting in 2005 for plasma derived factor VIII, that risk assessment had a sensitivity analysis, which gives you an idea which inputs to the risk assessment most affect the output or the level of risk.

Indeed, the clearance of TSE agents during the manufacturing process is one of the major things that did impact the ultimate estimated risk to recipients.

However, standardized methods for studying TSE clearance in products have not been defined, in part because there are a great number of challenges associated with standardizing these methods, since we don't know everything there is to know about the TSE agents in blood.

We seek your advice about whether standardized methods and assessment criteria are feasible now, and if they are appropriate for determining TSE clearance in the

manufacturing processes for plasma derived factor VIII products.

In particular, these are your three items for discussion, the feasibility and scientific value of adopting standardized methods to assess TSE clearance in manufacturing of plasma derived factor VIII products, and whether a minimum TSE agent reduction factor might reasonably serve as an appropriate standard for demonstrating vCJD safety of plasma derived factor VIII products.

If there is a minimum level that might reasonably serve as such, what action should FDA consider if only lower levels of clearance can be demonstrated for a given factor VIII product.

I am going to go into a little bit of the previous history of TSE clearance studies and FDA's involvement in those.

Some of the members here today might remember that we discussed TSE clearance studies with you in February of 2003.

Since then, we have engaged in case by case review of the following types of information on TSE clearance. So , this is information submitted by manufacturers officially to FDA, requesting a labeling claim based on specific data that they had generated.

These studies include a rationale for the animal model selected and a rationale for the spiking preparation. I will get into some of these details in a minute, characterization of the spiking agent, demonstration of accurately scaled down processes, robust and reproducible experiments, well characterized assays for TSE infectivity.

These submissions will contain estimated logs of TSE clearance by the processing steps that were studied, and also will demonstrate or describe mass balance, that is, accounting for all the input infectivity in the output samples that are assayed.

There should also be a demonstration that mechanically similar clearance steps are or are not additive in the process, and an accounting for conditioning of infectivity.

By conditioning, what I mean is, a prior step in manufacturing might affect the physical state of the TSE agent and, in turn, impact the amount of clearance that can be effected downstream in the actual clearance step that is being studied. Again, I will go into this in more detail in just a minute.

Since that time, four labeling claims have been approved. These are for Carimune NF and panglobulin NF. These are immune globulin products, and these are the reduction factors and these were the steps that were

studied, precipitations and nanofiltration for these two reduction factors of 7.2 and 4.4.

Gamunex, another immune globulin product is where a combination of cloth and depth filtration were studied and the clearance level obtained was an average of 6.6. Thrombate III, precipitations were studied with a reduction factor of 6.0.

Now, you notice there aren't any plasma derived factor VIII products here. That doesn't signify whether we are evaluating submissions or not, but it does tell you that no such studies have been approved for labeling claims.

Why are we particularly concerned about plasma derived factor VIII? Well, we are concerned, of course, about all plasma derivatives, but cryoprecipitation is the first in manufacturing of plasma derived factor VIII.

There are many other steps that may follow as there is increasing purification to make the product. Dr. Kreil will be talking about those more.

This is just a schematic of Cohn-Onclay blood plasma fractionation process. What you can see is the cryoprecipitate which becomes plasma derived factor VIII comes off very early in the scheme.

So, one of the reasons to be concerned is that there is not much opportunity for clearance like there

might be for albumen, which undergoes a series of sequential precipitations to be purified, and immune globulins where the case is similar.

Many people have looked at experimental clearances, either PRP<sup>TSE</sup> or infectivity by cryoprecipitation, and this is just some of the references that have reported this.

You can see here that the log 10 reduction factor is really one log or less in all of these studies, and whether a bioassay or a binding assay was used of a surrogate marker for infectivity.

So, these are the major challenges that we face in standardizing these studies or even understanding how best to do these studies.

I am going to talk about four things: the exogenous or spiking experiments, endogenous experiments and their relevance and feasibility, the TSE strain and animal model that is used, and output measures of infectivity reduction -- bioassays, which are infectivity assays in animals, and in vitro assays such as the western blot and the conformation dependent immunoassay, which depends upon antibody binding.

This is a very simple schematic of how an exogenous TSE clearance study might be done. The example I have given you here is the cryoprecipitation steps.

So, here you would have plasma. You would spike a preparation typically from brain of an infected animal into this plasma, and then that would undergo the manufacturing step where you get cryoprecipitate and cryopoor plasma supernatant.

The infectivity would be measured here and here and compared to the amounts that you put in at the beginning to determine a reduction factor.

So, for right now, I am going to be talking more about spiking experiments. There are a number of studies that have been done this way.

The reason that spiking experiments are done with brain homogenate is that it tends to have a very high infectivity level. So, you can achieve or demonstrate a wide range of clearance values. It is practical.

The form of the infectious agent, a number of these have been used, including membrane associated forms, brain homogenate that is just centrifuged to clarify, ultra centrifuged preparations including microsomal preparations and coevally like domains.

People have also used detergent solubilized hemogenates from brain. There are also membrane free infectious materials that have been used.

Again, they are derived from brain. These would be fibrole preparations, but also more purified

preparations of PRP<sup>TSE</sup> .

In general, they can be somewhat insoluble and they are felt, perhaps, not to be the best representatives of blood infectivity, which is believed to be more soluble.

Here I am just going to show you some examples of what we know about the spiking form of the agent. This is by Bey et al, published in Biologicals.

This is just to demonstrate that the form of the spike impacts clearance by precipitation. What you see here is the manufacturing process, cryoprecipitation, two different alcohol precipitations, and glycine precipitation.

These are the logs of PRP<sup>TSE</sup> reduction that were measured with respect to the supernatant. This is microsomal spikes. You can see that, for cryoprecipitation and glycine precipitation you have a fairly low clearance.

If you look at a more purified PRP scrapie spike preparation, you get somewhat higher levels of clearance, here two, three and four.

So, spike has a major impact on the amount of clearance that you measure, which means it is very important to choose, if you will, not necessarily the worst case spike, but perhaps the one that might be most representative of infectivity in blood.

There is also the impact of conditioning. This is

an example of conditioning, where detergent treatment of the infectious preparations diminishes its clearance by nanofiltration. This was published in 2001 by Tateishi.

The feed solution is a starting solution. So, this brain homogenate was treated with detergent or without detergent, and these are the titers of infectivity that they got.

By the way, these experiments were done by bioassay. So, all of these output measures are amount of infectivity by bioassay.

So, this is what you begin with, the detergent and non-detergent treated material. That was put through a 35 nanometer filter.

What you can see here is that material, brain homogenate, that was not treated with detergent had a very nice clearance by nanofiltration, 35 nanometers.

When detergent was added -- I think this is sarcophil three percent, you get a much lower level of clearance.

So, what does that mean? That means that if you have a detergent step, that that might impact the filterability of the agent at the end.

What you can also see is that, at lower pore size filters -- that is, 15 and 10 nanometers -- you do get good filtration in either case.

What this means for TSE clearance studies is that you really need to consider the upstream steps that might impact the agent.

You could get, in a sense, a result that might not really reflect, if you only looked at nanofiltration and not the upstream steps before, the impact on the agent, it means that you might over-estimate the amount of clearance, for example, by nanofiltration.

This is one of the complexities of doing these kinds of studies and one of the challenges faced by industry.

Another example of conditioning is PRP<sup>TSE</sup> clearance by membrane filtration and depth filtration. This was shown in a paper by Van Holten, to increase in the presence of alcohol.

It appears, from his data, that the alcohol the used and the concentration of alcohol caused aggregation of PRP<sup>TSE</sup>, which obviously influenced how well it was filtered by fairly large pore sized membranes and depth filtration. It was clearly filtered much better in the presence of alcohol.

I am going to briefly review the endogenous infection model. In these models, plasma is taken from a TSE infected animal.

This is very low titer material, somewhere on the

order of a couple of IDs per ml to maybe 50 or 60, depending on the animal model used.

It undergoes the manufacturing step again, and you measure the amount of infectivity in the supernatant in this case, or the precipitate.

The endogenous TSE clearance studies have relevance to blood infectivity. I would point out, though, that the comparison of results from endogenous and exogenous infectivity studies suggest similar reductions for some precipitations, but the number of comparative studies is extremely limited.

However, endogenous infectivity is probably the most relevant to infectivity that we would find in human plasma.

The characteristics of endogenous infectivity are thought to be a fairly small size of the infectious particle, difficulty in sedimenting in its native form -- and by sedimenting I mean by high speed centrifugation. It is probably poorly aggregated and it may be lipid or plasma protein associated.

The relevance to human blood is highly likely, but you can only demonstrate limited clearance because the starting infectivity is so low.

That means that a large number of donor and assay animals have to be used to compensate for these low titers.

So, in other words, if you have two infectious doses per ml, but you assay 100 mls of material, you will likely find this infectivity.

Just to give you an idea, the volume injectable intracerebrally to titrate this is only about 20 microliters -- sorry, .02 mls at 20 microliters per ml, or about 50 microliters per hamster. For 100 mls of plasma, to completely titrate it, you need 5,000 mice or 2,000 hamsters.

What is done in real life, I don't know if anybody has ever done quite this many, but they will do a portion of their output sample and look at infectivity and calculate essentially how much they could have missed if they get a zero.

Large animal models, in theory, might be nice to study. We know that sheep can have natural infection with scrapie and can be experimentally infected with BSE.

We further know that the blood of these infectious animals is infectious to recipient sheep. There are experimental logistical hurdles in doing these kinds of studies

. Among those are herd management, and the fact that there would be very limited locations where you would be allowed to have a scrapie herd or a sheep herd infected with BSE. Furthermore, these would have to be very

carefully segregated away from the control animals.

These sheep also have very long incubation times. So, you would have to wait even more years than you would have to wait for a hamster or mouse study and they will be limited in availability.

Of course, the logistics of scale down would be different because you would have much larger volumes to work with, but you would still have to use a pilot laboratory to simulate the manufacturing process. Probably new or different pilot laboratories would have to be set up to study clearance in large animals.

In terms of TSE model selection, there are many papers that show that TSEs differ in their resistance to inactivation but, to date, clearance of TSEs in plasma products has only been demonstrated by partitioning studies.

The reason for that is that the inactivation methods that have been used for TSE infectivity are very harsh methods and you would destroy the proteins that you are trying to isolate and purify for people to use by any of these methods.

There are very few direct strain comparisons in TSE clearance study plasma derivatives, but alcohol precipitations were looked at by Stenlin(?) and the group at Telecris. They found similar clearance levels using

western blot for BSE, CJD and vCJD spiked samples.

Alcohol precipitations, at least in theory, could be influenced if strain related differences exist in aggregation properties of the infectious agents.

This is a theoretical concern, but it might also be a real concern, and we don't have the data to know whether or not that is the case in these particular scenarios.

So far, strain differences for partitioning clearance experiments have not been demonstrated. You can see how limited in number the studies are.

What kind of assays should be used for TSE agents in clearance studies? Bioassay is usually done by limiting dilution titration into susceptible rodents and, as you have already heard today, PRP<sup>TSE</sup> is felt by many to be a good surrogate marker for infectivity, and this is usually measured by western blot or conformation dependent immunoassay, as you have heard.

There is a rationale for retaining bioassay use, because although binding assays detect PRP<sup>TSE</sup>, they are examples of infectivity without detectable PRP<sup>TSE</sup>.

I should qualify that by saying very often this has been PRPres, that is, PRP<sup>TSE</sup> as assayed by its resistance to proteinase K.

There are also examples of PRP<sup>TSE</sup> occurrence

without infectivity and also I would note that conditioning that I have shown you before might differentially affect binding versus infectivity.

There is a paper by Silvera and his group suggesting that, at least from brain homogenase, a certain size of prion particle seems to be associated with greater infectivity, and that larger aggregates and smaller aggregates are less associated.

So, even in the context of the protein only hypothesis there are some caveats that one would have to consider in terms of using PRP<sup>TSE</sup> binding solely as a surrogate for infectivity.

Furthermore, binding assays currently are not as sensitive as bioassays. We have just heard, however, that for the conformation dependent immunoassay, this may be otherwise, and we look forward to additional data in that respect.

The limit of detection for binding assay, more typically, is two to three logs of infectivity. So, you can't demonstrate as wide a range of occurrence when you are using, in general, these kinds of assay.

An additional challenge in TSE clearance studies is their interpretation, how much clearance is significance. Well, we are going to ask the committee to discuss that, but I can give you something to work with.

In viral validation or viral clearance studies that are done for all plasma derived products in the United States, it is typically demonstrated for effective viral clearance that there are at least two to three logs greater clearance than the maximum potential absolute amount of virus present. When I say the amount of virus present I mean the amount of virus that would be expected in infected plasma.

This added margin of safety is probably important, because we don't know in every case how much virus might be in infected plasma. We have a very good ballpark estimate based on what is reported.

Furthermore, manufacturing itself has its -- is not entirely robust. You might not get exactly the same value each time you do a manufacturing step because of slight changes in parameter.

Again, this margin of safety, this addition of more logs on top of what you think absolutely has to be removed, is probably a good idea.

Now we come to TSE clearance. If TSE infectivity is present in a unit of plasma, how much might there be. Well, if you take an 800 ml plasma unit -- this would be the top amount you might expect, and multiply that by the potential infectivity in it -- and we really don't know exactly if these numbers are right -- so, these are

estimates based on other studies in the literature from animals.

So, you multiply by this range, two to 30. What you get is 1,600 to 24,000 range of infectious doses possibly expected in this infected plasma.

That works out to 3.2 to 4.4 log 10 total infectious units. Actual infectivity might be less than this due to the blood brain barrier and due to host susceptibility, but this gives you some numbers to start with and to think about.

I am going to introduce the questions, but Dr. Kreil will be following up with additional thoughts about TSE clearance studies and more detailed information about where industry has been studying clearance in plasma derived factor VIII.

We are asking you to comment on the feasibility and scientific value of adopting standardized exogenous or spiking study methods to assess TSE clearance in manufacturing of plasma derived factor VIII.

We would like you to comment on your thoughts on optimal spiking material and its preparation from the standpoint of relevance to blood infectivity, the selection of TSE strain and animal models.

TSE immunoassays for PRP<sup>TSE</sup> versus bioassays for infectivity, the use of these as output measures, and

identification of manufacturing processes that might alter TSE agent properties.

We would also like for you to comment on the feasibility and scientific value of adopting standardized endogenous study methods to assess TSE clearance in plasma derived factor VIII.

We would also like you to discuss whether a minimum TSE agent reduction factor demonstrated using an exogenous spiking model in scaled down manufacturing experiments, like the ones I have described, might reasonably serve as an appropriate standard for demonstrating TSE safety of the products.

Considering the outcomes to that discussion in question two, in cases where only lower levels of clearance can be demonstrated for plasma derived factor VIII products, what should we consider:

Labeling that would differentiate the lower clearance products from other products with sufficient TSE clearance;

Recommending addition of TSE clearance steps to the manufacturing method; Performance of TSE clearance experiments using endogenous infectivity models, or any other actions.

I will leave that with you and give the podium over to Dr. Kreil. Thank you very much.

DR. TELLING: Are there any questions for clarification for Dr. Scott at this time?

MR. BIAS: Dr. Scott, is there a reason that there haven't been any experiments done using human blood of vCJD victims?

DR. SCOTT: That is a very good question. I think that if this was easily available, they definitely would have been done.

I think by the time the patients come to their clinical disease, the ability and the ethical constraints on collecting a lot of blood or plasma from them has been limited.

In the United Kingdom, they are particularly careful to assure that patients have a choice and that their families have a choice.

That is what has caused the limitation. It is not obviously the patient's fault. There aren't very many patients to begin with, but there aren't very many people with this disease at any given time that are in a situation where they might be able to give a large amount of blood or plasma.

I know Dr. Minor is in the audience and I wouldn't want to just call on anybody in the audience at random, but either Dr. Asher or Dr. Minor might have more insight on the availability or the potential availability

of variant CJD blood. I guess I will let Dr. Asher, because he is on duty here.

DR. ASHER: I hope that we will hear some thoughts on the issue tomorrow from Dr. Minor who is here, and Dr. Turner who we expect to arrive this evening.

The problem has been this. With sporadic and familial CJD, infectivity has not convincingly been demonstrated in the blood.

So, if you collected it, either by epidemiological look back studies -- and the American Red Cross' study is really now quite extensive -- and a very limited number of studies done at the NIH transfusing whole blood into chimpanzees, none of whom ever became ill with Creutzfeldt Jakob disease.

So, the blood from the forms of Creutzfeldt Jakob disease generally available in the United States, the hypothesis that there is enough infectivity present to be detected at all with any of these assays has not been demonstrated.

With variant Creutzfeldt Jakob disease, as Dot pointed out, the number of patients available has been very small.

In the two cases in the United States, I believe that Dr. Gambetti has a small amount of blood, and I know the Canadian case there is a small amount of plasma

available, but nothing approaching what would be needed for the kinds of studies that we have been talking about.

I am afraid at the moment we are stuck with blood from endogenous infectivity. We are stuck with blood from animal sources.

DR. GESCHWIND: So, at UCSF we have actually shown that it is pretty feasible to get large volumes of blood from patients with CJD.

We have -- Jiri Safar probably can give you the fact numbers, but probably we have over 50 patients in whom we have gotten 200 to 400 mls of blood.

So, bring in patients from around the country and at certain points when we have funding we have been sending out a nurse to get 200 mls of blood from patients with CJD, and we have been collecting it every two to three months from patients during the course of their disease, depending upon -- we do very strict safety tests that are more conservative than for the Red Cross blood donations, prior to doing this.

So, it is feasible, particularly in patients whom we have diagnosed earlier in the disease course, and in patients who have a slower course.

DR. SCOTT: I think that Dr. Minor also has a comment maybe about the variant CJD cases.

DR. MINOR: Well, I am very jealous of the

comment that has just been made. I have discussed this extensively with the people at the CJD surveillance unit in Edinburgh, and they won't touch it.

They basically say that the ethical concerns are such that they will not take a unit from people who have variant CJD, no matter who wants it.

I will be talking a little bit about human samples tomorrow in the diagnostic presentation, and the availability of human samples is absolutely tiny, relevant human samples, like within the United Kingdom, is absolutely zero.

There has also been a recent introduction of a thing called the human tissues act, which means that if you don't do it right, you get sent to prison. That has actually been a major inhibitory effect on actually trying to get these kinds of samples.

I am actually very impressed by the fact that you can get those kinds of volumes around. If we could get those kinds of volumes, I think I would put them into diagnostics rather than into plasma fractionation, frankly.

DR. TELLING: What about blood from BSE infected cattle?

DR. MINOR: This is like experimental infections you are talking about or what?

DR. TELLING: Either experimentally infected --

well, presumably that would be the most convenient source.

DR. MINOR: Again, I will talk about some of that stuff tomorrow. There is a study which is going on with Ferna(?) Huston on sheep, blood transfusion, where I think this is actually a kind of interesting animal model for this.

The idea is that the sheep will be infected by mouth by BSE or whatever, and then blood will be taken from them and transfused into other sheep which are negative.

If you can actually keep a sample of the blood which is transfused, and you can also follow the blood samples from the transfused sheep -- I don't now how confusing I am making this sound -- you can start talking about when the diagnostic tests become positive and when they become negative.

You can also in theory, I guess, use those kinds of materials for fractionating plasma proteins. I think the wrinkle to that is it is not clear to me that plasma proteins fractionate from sheep plasma in exactly the same way as they fractionate from human plasma.

So, there may be a doubt about even the relevance of the model. I am sure that can actually be done. I am not sure that has been done, but I think it can be.

DR. TELLING: Any further questions?

DR. SAFAR: This is more a comment or offer. I

think that starting with Jim Mastrianni, who is at the table, and followed by Michael Geschwind, it was a very difficult and challenging project, the logistics and technical issues and the protocol issues.

With the help of NIH, with Michael Nunn and many other people who cooperated, I think that all of those logistical issues -- and that is an answer to Phil Minor more than anybody else -- it can be overcome.

It took time and it was really difficult, but I think that it is feasible to collect a significant amount, two ml, 200 ml, at a session from CJD patients, either variant CJD or sporadic CJD.

So, I think that this is one of the issues which should be discussed tomorrow in more detail, how to organize such a collection and how such a repository should be handled, funded and organized.

DR. TELLING: With the obvious caveat that sporadic and variant CJD may differ radically in their biological properties with respect to infectivity in blood.

DR. SAFAR: Absolutely, yes.

DR. MANUELIDIS: I would like to make one comment about sort of the definitive comment that David made about sporadic CJD.

It was shown in guinea pigs in 1978 that the blood is infectious and the spleen is infectious. It only

makes sense, really, that it would go to spleen if the sporadic CJD, the agent itself, went through blood.

The second thing is that there were two studies that were published in Lancet, one by our group and one by Tateishi's group, showing that human blood actually transmitted as well.

Now, the Japanese group might have a slightly different variant of their CJD because of the different geographic region.

I think that probably the amount of infectivity is much lower than it is in vCJD, but it is likely to be there, from everything we know about these infections and the fact that spleen is infectious.

DR. ASHER: We agree that in blood of patients with sporadic CJD, infectivity is likely to be there, but the amount would certainly have to be smaller.

Over 100 patients who received blood transfusions from donors subsequently confirmed as having CJD followed for more than five years by the American Red Cross, none of them came down with CJD, whereas a very small number of recipients of blood components in the United Kingdom -- 18 -- got presented, three have already come down. So, it is clearly a very different situation.

Now, I suggested for variant CJD that cadaver blood for some purposes would be satisfactory, but the UK

authorities, apparently found that idea distasteful. You can get more than a liter of blood from a cadaver, and privacy rights end at death.

DR. TELLING: Thank you. I think we had better move on to the second presentation. Dr. Kreil, industry TSE clearance studies for factor VIII.

**Agenda Item: Industry TSE Clearance Studies.**

DR. KREIL: Good morning, ladies and gentlemen. On behalf of the pathogen safety steering committee of the Plasma Products Therapeutics Association -- that is the group that I am going to talk on behalf of today -- I would like to thank you for giving us the opportunity to comment on some of the aspects that Dr. Scott has raised in her presentation just a little while ago.

This is just to remind you basically of our constituency, worldwide manufacturers of plasma derivatives. Specifically the considerations here focus on one class of products. Those are the plasma derived factor VIII products.

The manufacturer, just to give you a schematic of that, actually commences very early onwards from the plasma. This is thawed up to just a little above freezing temperature, at which point, in plasma, there is a precipitate that forms, the so-called cryoprecipitate.

By centrifuging these, precipitates can be

removed from plasma and that way you basically split up plasma into two fractions, one being the cryosupernatant here, for the production of certain coagulation factors, but then for also the classical Cohn products, Igs and albumen.

Then the cryoprecipitate, where factor VIII products are manufactured, historically they have been turned into high purity. That is meant to say that, beyond factor VIII, they may contain albumen factor in addition.

This is the principle of how TSE or, for that matter, all virus or prion clearance studies are being performed.

So, you have a very large scale manufacturing process. Typically we are talking thousands of liters. Obviously, you cannot work with pathogens at that level for GMP considerations to start with.

So, what is done is, we are scaling down these processes into a scale that we can work with these processes in what we call pathogen safe laboratories.

There we can work with biosafety level agents, and what we do basically is, we run these processes at a very small scale, typically upstream with a little less pure intermediate, a little larger in volume, and then running through one of these purification steps.

What you get is typically a smaller volume

intermediate of higher purity. Now at the laboratory scale, what we can do is, we can add upstream, for the purposes of today's discussion, prions but, again, we are doing the same thing with all sorts of viruses also.

Then, after this is added, you go through this manufacturing process at the laboratory scale. Then you can determine the input of prion activity or prion surrogate markers and the output.

Then, by comparing that, and taking into consideration the volumes before and after, you can actually derive what is the so-called reduction factor, so you can understand what reduction of prions for that purpose is achieved by that manufacturing process.

A very important point obviously is, this needs to be exactly like this. Otherwise, the numbers that we obtain are not meaningful for the large scale production of biological medicinal products.

That is why a lot of time is spent, once you have established the so-called downscale, into validating that down scale.

Really what we mean by validating that down scale is that we are going to validate that this downscale is equivalent to the large scale manufacturing process, because this, again, is the fundamental principle under which we can derive knowledge about the large scale

processes, from doing these small scale experiments.

So, what can be done to make sure that the information that we derive at the small scale is meaningful? Well, first, the intermediate that we use for running these small scales is directly derived from our manufacturing facilities, or is somewhat specialized materials and we get them maybe from a pilot scale.

In other words, this is regular intermediate that would be manufactured into commercial product. Now, for this product, a number of different parameters can be assessed, not only for the input, which is equivalent anyway by means of its origins, but also, after doing the small scale purification, you can determine whether the amount of protein concentration or activities, for the purification that supposedly should occur in the large scale, does also occur in your small scale.

Further, a number of process parameters can be monitored and, as you can see from the numerous examples on this slide, these do vary depending on the step that we investigate.

If it is a precipitation step, then obviously the concentration of the precipitating agent, the time that the intermediate is stirred with this agent, the temperature would be important.

Then, for example, calling for other steps, then

things like pH, conductivity, ionic strength or contact time with the resident would be more important.

Again, this is sort of the prerequisite under which we have to operate. So long as you can't demonstrate that there is a perfect equivalent of the small scale with the larger scale, all further information that you derive would be meaningless for the large scale.

Now, for prion clearance studies specifically Dr. Scott has pointed out already that there are a number of choices that one needs to make before going into a prion study.

There is first the choice of a spiking agent from which organism we want to derive that. Secondly there is a number of different possibilities for particular spikes for the initial spike preparation.

Initially people have used the organ that does contain typically the highest levels of prion levels -- the brain -- and have more or less purified that as a spiking material.

So, brain homogenous has certainly been the first material used, and then different purification forms off that, such as microsomal fractions, detergents treated or sonicated intermediates of that.

Finally, there is a choice of which assay you want to use for a readout of your prion clearance study.

That would be either in vivo, which is cost intensive, resource intensive, and you will have to wait a long time to get a result or, alternatively, in the in vitro assay such as the western blot or the CDI.

Now, it is important to understand that, for these prion quantifications, there are a lot of controls also put around this such as, for example, physical reactions are quality control.

There are good laboratory practices applied and, where it is not possible to become certified for the application of good laboratory practices for such studies, and that, as I said, is not possible in all geography. Then at least the principles are being followed.

Then finally, the preparation of the spike materials and how the assays are performed, when an assay is acceptable or not.

All of this is writ down in what we call standard operating procedures so that there is a lot of control being put around the reproducibility of these assays.

Obviously also, such as with every good assay, controls are being put in place, such as a positive control, negative controls, controls for interference of the matrix with the performance of the assay, et cetera.

So, I guess we can say that, by putting in place all of these controls, we can certainly guarantee that the

assay is suitable for the purposes of a prion reduction study.

The agency has asked the question whether further standardization or a validation of such assays would be useful, and we would like to make a number of arguments why we believe that that would be useful.

First, I have to mention that, during these manufacturing processes, it can be observed that the initial spike is being conditioned through the process, such that actually you would want to use different materials for investigating different manufacturing processes.

If, for example, you had a solvent detergent treatment upstream from the process that you want to investigate, then any prion that would have been present in plasma, that would have come down to the step that you investigated, would have gone through that contact with solvent detergent.

So, it might be a chance to consider using a spike that has been detergent treated. That would reflect, probably most adequately, whatever was upstream in the process.

Also, investigating the potentially additive effect of sequential steps will require you, for example, to do a run without subsequent spiking. So, again,

standardizing how exactly you need to do that experiment is going to make the interpretation difficult and will limit your ability to demonstrate removal.

That is why we would argue that it is more important to rely on good expert judgement first, and then obviously also justification of that judgement on a case by case basis.

Another useful example, we believe, to look at this -- and again, Dr. Scott has shared with you the very same example that I will bring up here -- it has been shown that there can be very substantial removal here by log steps, for example, using a filter with a nominal core size of 35 nanometers.

In the presence of detergent during the preparation of the prion spike, however, that removal becomes less significant, and on the smaller core sized filters, we have been able to remove the spike.

Now, further data that are available and yet unpublished have shown that if you use more drastic detergent treatment and sonication of the prion spike material, then in reality you can get the material even through a 15 core sized filter, with virtually no removal at all.

So, one might argue that that would then be a worst case and such a spiking material should be used for

the evaluation of nanofilters.

So, our belief is that these conditions are certainly very important in trying to understand the elementary nature of the infectious unit for prions, but I think these should be seen as experimental conditions.

During manufacturing, we do certainly not add these high levels of detergents, and certainly we do not sonicate our intermediate.

Therefore, should any prion agent be present in plasma, then it would not be sonicated detergent treated to the degree that has been used in this more experimental set up, to understand better the nature of the agent.

That is why we believe that the reduction capacity for nanofiltration has been widely demonstrated under more relevant conditions for manufacturing.

There are some recent, I would like to call it, advances in science that would suggest that maybe we should be using different spiking materials to the ones we have used so far.

A very recent piece of evidence has come from an Italian laboratory where it was demonstrated that starting from brain homogenate at a titer of roughly  $10^8$  infectious units per ml, after a very high spin, you can actually device material in the supernatant here, and that supernatant still has a very high level of infectivity, yet

very little or even no PRPres demonstrated by, for example, western blot.

That paper, in the conclusion this should suggested that there should be a suitable spiking material to use in validation.

Now, some of the limitations there already are, that you would only be able to do in vivo assays because if there is no PRPres even in the starting material, then that would not be a good readout for a reduction study.

Another complication, however, is that this  $10^5$ , while still a reasonable titer, represents only one-thousandth of the original input. So, it is a tiny little minority of the original PRP agent.

Now, that we have seen already in an earlier study, where endogenously present infectivity has been fractionated using a less drastic centrifugation here.

Even for endogenous or, if you will, the relevant former sensitivity, you can see that with centrifugation you can pellet quite a bit of that infectivity.

So, the question becomes, if you are looking at these infectivities, are you interested in the majority of the infectivity or do you want to investigate a tiny little minority that, in behavior, may not at all reflect what would be present even in an endogenous infectivity situation.

Another piece of recent information, a very elegant paper has been published recently that I would like to discuss because it seems to have pertinence to the conduct of reduction studies.

It would be an experimental model, a transgenic mouse model, where these might express PRP without the GPI anchor.

Now, if these mice are infected with prions, then they do not develop a classical pathology of scrapie. What is interesting is that they have very high levels of infectivity circulating in their blood.

One might argue that that would be a high titered blood spike so something rather usable for validation studies. Certainly this has been suggested to be the case by the authors.

I guess one argument that I would like to convey to you is that this PRP protein is devoid of the GPI anchor and, therefore, this truncated version is of unclear relevance to the pathophysiologically relevant prion agent that we are concerned with, should it really be demonstrated to occur in plasma.

I would argue, if we investigated the removal of this truncated form, then those results might, because of the similar or dissimilar nature of the agent, tell us something about the true agent or might not tell us

something.

In summary, we feel that certainly through the validation of equivalence between large scale and down scale, the controls we put around all the prion spiked materials and also the controls that we put around these prion assays, prion clearance studies as we have performed them up until now certainly have generated meaningful information.

We feel that, therefore, further standardization would, in fact, inhibit process specific investigations more than anything else.

We feel that we should more rely on expert input, obviously providing the adequate justifications. Further, given the enormous advances that science comes up with at a very rapid pace -- the two papers that I have just shared with you have actually been published in the last two months only -- would also prevent using novel approaches that might allow us to investigate more meaningful processes, and I think would really discourage, more than anything else novel approaches.

As Dr. Scott has mentioned, we are now going to share with you a summary of different prion clearance studies that have been performed throughout the industry on specifically plasma derived factor VIII products.

Before showing that to you, I would like to make

a number of qualifiers. You need to keep in mind that not all of these products are manufactured using the same manufacturing process.

This also results in different clinical usability, such as some of these products contain non-relevant factors in addition to factor VIII and therefore cannot be seen as typical or just another factor VIII product, if you will.

Also, for the reduction factors that you are going to see for the overall clearing, it is not necessarily so that all the manufacturing steps have been investigated.

So, a lower clearance factor may just mean that not all of the steps have been investigated. Should that be done, the numbers could be much different.

Also, we would like to point out that, for the products that have been licensed in the United States -- and that is mentioned in the footnote of the slides -- these data have been shared in more comprehensive fashion with the agency.

We would also like to point out that, at this point, there are a number of research studies going on. The results we will await and will provide further results.

So, this is the first one. We have taken a look at two manufacturing processes that have been investigated,

one being purification with a monoclonal antibody column, and then there is another ion exchange chromatography column.

This is since the 263K strain of scrapie adapted to hamsters have been used, with an infectivity assay. So, bioassay was used for generating the numbers here. You can see a total log reduction of roughly eight logs was demonstrated.

Here is another product, also licensed in the United States. Here four different manufacturing processes have been investigated -- actually three, I apologize. That is a PG precipitation here, another affinity chromatography step here, and then a final precipitation plus final filtration.

What has been used here are two different preparations of spike, one being a microsomal preparation here and a detergent 3-D preparation here. Here, the same detergent 3D preparation with the brain homogenate as a complement, and again here, the microsomal and the detergent preparation.

Two independent runs have been performed first by preparation and the mean reduction factors you can see down here. The product overall has a demonstrated safety margin of greater than nine logs of prion.

Another product here has investigated two

combinations of steps, one being a sequence of precipitation procedures, and the other a sequence of chromatography events.

There have been, in this instance here, two spike preparations used, here one, and two independent ones, first by preparation, have resulted in these reduction factors here. It should be pointed out that this product is not licensed in the United States.

Another product from the same company, again not licensed in the United States, again, sequential procedures have been investigated here.

Here the spike preparation is mentioned here, in a single run, again using the western blot in vitro assay, if you will, with a cumulative roughly six log reduction for these products.

Company D, that product is also licensed in the United States. Again, two different spike preparations have been used, a purified PRP<sup>SC</sup> or microsomes.

It has been assayed with the CDI assay and two runs were performed per spike preparation, resulting in these mean log reduction factors here.

A further product that is licensed in the United States, here is the sequence of events, if you will, when one goal has been investigated. So, sequential steps.

This has been done with two different spike

preparations, and one run per spike preparation was performed with a mean reduction factor of 3.7 to 3.8.

Finally, this is the last product, again not licensed in the United States. Here, again, another sequence of steps has been investigated with a single spike material, brain homogenate, and that resulted in a 3.5 log reduction.

So, summarizing all of these already summarized data, I would like to point out that we feel that manufacturing processes for plasma derived factor VIII products do remove prions, to varying degrees.

The individual reduction factors that we had on the summary slides really depend on, first, the specific manufacturing process. That is also resulting in different product quality, if you will. Secondly, obviously these numbers depend on the number of steps that have been investigated. The more steps investigated, the higher the numbers.

Finally, to some degree, on the experimental design. Using in vivo assays, for example, allows you with a higher dynamic range to demonstrate larger reduction factors. So, there may just be larger reduction factors inherent to the assay system that you use.

In summary also we feel that, in terms of the safety margins of these products, it is important to point

out that the level of risk at this point remains unknown, the specific level of risks, but very likely the level of risk is low.

There is not any evidence for the transmission of prion diseases by plasma derived factor VIII products, and that despite the very high level of pharmacovigilance, I would like to mention the multiplication exercise that the United Kingdom has gone through.

Patients, where it is known that their product has been derived from also the contributions of latent bearing(?) CJD donors have been notified of their presumably increased risk, and these people are being closely monitored.

Epidemiologically, I think it is also important to point out, as Dr. Scott has also mentioned, that the exposure is low and the exposure seems to be getting lower still.

There is, as I did hopefully convince you, a reduction of prion agents by all the plasma derived factor VIII manufacturing processes that I have shown to you.

Therefore, we feel that the quantification of reduction versus an unknown certainly low level of risk is an open equation at this point, really.

In conclusion, we would like to say that, given the unsubstantiated level of risk associated with plasma

derived factor VIII, we feel that this is not a rational basis for implementing further measures, because it needs to be kept in mind that any additional steps that might be implemented might also adversely impact the product characteristics, starting with clinical safety, but then with additional manufacturing steps, also typically yields suffer and, therefore, availability may be affected.

I can say on behalf of industry that certainly we continue to be committed to research. We have done these studies on a voluntary basis and, as I said, further studies are currently being conducted and results will be made available. Thank you very much.

DR. TELLING: Thank you, Dr. Kreil, for that perspective. Are there any questions at this point, or comments?

DR. HOGAN: Tom, I can assume, then, that the products, after these additional steps, are all biologically active and have been evaluated for safety?

DR. KREIL: The steps that I have summarized for you this morning are the steps that are being conducted during the manufacture of commercially licensed product.

So, all of these products are clinically usable because they wouldn't have received a license otherwise. It is just that history has shown that, whenever manufacturing processes are changed, such that, for example, greater

virus reduction is afforded, that typically that results in a reduced yield of these product, and clinical usefulness of the product then needs to be reestablished by clinical trials.

DR. HAMILTON: What concern is there about the exceedingly long incubation period for CJF and variant CJD, and also could you speak about leukoreduction effectiveness?

DR. KREIL: Well, regarding the long incubation period of variant CJD, this is one of the aspects that still will not allow us to come up with a final judgement, I guess. It is one of the uncertainties. We just need to wait for further advancement and understanding of these diseases.

This is why we don't say that there is categorically no risk, because I think at this point we cannot say this.

In leukoreduction, leukoreduction has been very elegantly investigated for the reduction also, prion activity.

As far as published data, leukoreduction has actually been shown to just result in a marginal reduction of prion infectivity.

There is no further research going on to enhance this leukoreduction to provide the filter with an added

prion removal capacity but, to my knowledge, these devices are not yet available.

DR. HAMILTON: Could you compare this rarity or lack of -- this low incidence in this situation to the low incidence that was supposed in the early 1980s with HIV?

DR. KREIL: That is a very difficult comparison, I think. Certainly with HIV, very quickly, during the early 1980s it was realized that there was a blood transmissible agent there.

While the virus was not known at that point, research did quickly establish its presence, and actually the virus was present at very high levels, as we know.

To compare this with prion agents where, despite intensive research, the demonstration of presence in plasma has not been successful, I think would be very different.

I mean, certainly the levels of risk are very different. It has been mentioned today that, out of 18 potential opportunities for transfusion transmission of variant CJD, three have resulted in a transmission, which is very different from viral diseases as we know them.

There you would be looking at a 100 percent transmission likelihood, if we took the figures of blood and transfused it into a recipient. I think this comparison would not be appropriate.

DR. BROOKMEYER: could you comment some on the

reproducibility of some of those reduction factors of the data that you showed?

Some of the data, it looked like there was only one independent run or one or two independent runs. If you could just comment on the variability and also on how much you think those reduction factors depend upon the input and how much is actually being spiked in. What are the sources of error or variability in those reduction factors?

DR. KREIL: I was trying to point out that obviously we do everything possible to control these experiments well.

This is what scientists do. At the end of the day we like to have information that is meaningful. We are not trying to make up numbers, if you will.

Regrading the reproducibility, you are right that some of these experiments have been informed with an N of one, but others have been performed with numbers of repeats.

I can tell you that these repeats have been very reproducible. So, I certainly do believe that the studies as I have told you have been controlled adequately to ensure that the numbers are, first, reflective of what occurs in large scale and, secondly, have been adequately controlled so that the numbers are meaningful.

Now, all the limitations, all the caveats that

Dr. Scott pointed out are acknowledged. I mean, we haven't seen the agents occur in plasma. Therefore, we don't know exactly what it would look like in plasma, should it occur there.

So, all the spiking materials that we are currently using are models. Therefore, the specific number with the agent, would we have it, as it occurred in plasma, would it occur there, might look slightly different.

I guess one important point to mention is, all these numbers are numbers on a log scale. So, if a number is 3.1 or 3.3 quite frankly, for all practical purposes, the same thing.

In a log scale, again, there are potencies of 10 that you are measuring against. So, minor differences that might occur during experimental set ups would be insubstantial versus the reduction factors that we have seen.

DR. TELLING: If there are no further questions, thank you, Dr.Kreil. Next on the agenda is the open public hearing. Bill, would you let us know who is registered for the open public hearing?

**Agenda Item: Open Public Hearing.**

DR. FREAS: As part of the advisory committee procedure, we hold open public hearings so that members of the public can address the committee on issues pending

before the committee.

Mr. Chairman, at this time, I have received four requests to speak in the open public hearing sessions, one request for this morning, two for this afternoon's sessions, and one for tomorrow.

I would like to invite the speaker for this morning, Dave Cavanaugh, government relations for the Committee of Ten Thousand, up to the podium.

While he is coming to the podium, Mr. Chairman, I would like you to read the open public hearing statement required for the meeting.

DR. TELLING: Both the Food and Drug Administration and the public believe in a transparent process for information gathering and decision making.

To assure such transparency at the open public hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement, to advise the committee of any financial relationship that you may have with any company or any group that is likely to be impacted by the topic of this meeting.

For example, the financial information may

include the company's or a group's payment of your travel, lodging or other expenses in connection with your attendance at the meeting.

Likewise, FDA encourages you, at the beginning of your statement, to advise the committee if you do not have such financial relationships.

If you choose not to address this issue or the financial relationships at the beginning of your statement, it will not preclude you from speaking. So, Dr. Cavanaugh?

**Agenda Item: Statement by Dave Cavanaugh.**

DR. CAVENAUGH: My name is Dave Cavanaugh. I am government relations staff for the Committee of Ten Thousand.

I think as many of you may have heard in one place or another, our president, Cora Dubin, likes the expression coined in the last year or so of having an arm in the game.

All during this discussion I have been sitting here and thinking we have all these studies that show that prions can be reduced in factor.

We don't have a lot of information that they are being reduced in factor. That factor that is going out now, the factor that is going into people's arms, is still the same factor that it has been.

Today's discussion of prion reduction through

fractionation brings little comfort to persons with hemophilia in the United Kingdom.

Different interpretations of the findings of science show the United States and the United Kingdom to be going down very different roads on this subject.

As long ago as 1999, when the FDA first announced that screening for classical CJD was no longer needed, the agency began distinctly identifying the greater perceived safety of plasma products to whole blood:

"...experimental studies in animal models for CJD suggested that manufacturing procedures used for plasma derivatives could lower the amount of infectious material present in plasma derivatives compared with whatever levels could be present in blood."

In 2002, FDA moved further down this road:

"...we recommend that you defer donors of whole blood and blood components intended for transfusion. source leukocytes and recovered plasma, but not donors of source plasma, who have resided in Europe for a cumulative period of five years or more, between 1980 and the present."

This exemption of plasma occurred when the whole blood geographic donor ban was first being expanded beyond the United Kingdom.

Since that time, there has been no retraction from this position by the agency. Thousands of units have

been collected throughout Europe, pooled and fractionated to make factor VIII, IVIG, albumen and other products, which of course by now have all been consumed, largely by Americans.

It was in 2003 and 2004 that true cases of vCJD transmission by blood were reported in the literature. From that point on, all of the language of prior government and industry regarding theoretical risk of transmission became obsolete.

The United Kingdom, following a different time line regarding discovery of the dangers of variant CJD in blood, learned of the contamination of plasma pools years earlier, and declared the recipients of products from those pools to be at great risk.

The now famous 2004 letter from the Ministry of Health to 4,000 homes of persons with hemophilia instructed them not to donate blood, organs or tissue, and to inform medical, surgical and dental providers, so that disposable instrument can be arranged for in advance of any procedures.

Two years have passed since the risk communication exercise in the United Kingdom. The stigma it brought to every family with hemophilia is somewhat dulled now, although it was unprecedented and disruptive for weeks at the time.

So, which is it? Are we at great risk or is there no risk, or rather undetectable risk, which is not the same thing?

CBER advisory committees are often asked to decide on issues for which there is inadequate data to make sound judgement.

COTT has watched this country's response to TSEs unfold, from the USDA's denial that there is any problem to the untracked venison eaters in areas of US CWD outbreaks.

We ask that you do not give ground. Do not expand the exemption from geographic donor bans which plasma collection now enjoys. We further ask that you retract altogether this dangerous exemption of source plasma from geographic donor bans. Thank you.

DR. TELLING: Thank you very much. Are there any questions or comments? Is there anyone else in the audience who would like to address the committee at this time?

Okay, I thank everybody. We can adjourn until 1:00 o'clock for some lunch.

[Whereupon, at 11:50 a.m., the meeting was recessed, to reconvene at 1:00 p.m., that same day.]

A F T E R N O O N    S E S S I O N    (1:05 p.m.)

**Agenda Item: Open Committee Discussion.**

DR. FREAS: Before we begin the afternoon session, Mr. Cavanaugh has asked to address the committee for a brief announcement.

MR. CAVENAUGH: I would like to correct some possible misinterpretation of what I said before, just the part about the dissimilar geographic donor ban between whole blood and plasma in Europe creating a lot of blood collected in Europe from countries that don't have the ban and brought here from processing. That doesn't occur, and I know that.

People who live there for some time, who are American, live over there six months or more, in many of the countries to which we now have bans, come back here and give plasma, and that is basically exposed to European risk factors. The other is definitely correct.

DR. TELLING: Thank you. Dr. Epstein?

DR. EPSTEIN: Let me just state it in my own words. I agree with what Dr. Cavanaugh just said. The committee needs to understand that plasma for fractionation into U.S. licensed products has never been sourced outside of the United States.

DR. TELLING: Are there any other comments? If not, thank you. So, the next item on the agenda is open

committee discussion. Dr. Scott, would you like to rephrase the questions for us?

DR. SCOTT: Would you like me to go through all of them or just one by one?

DR. TELLING: I think one by one for now.

DR. SCOTT: So, the first question is, we are asking you to comment on the feasibility and scientific value of adopting standardized exogenous or spiking experiment study methods, to assess TSE clearance in manufacturing of plasma derived factor VIII.

We are asking you to consider and to comment on what is the optimal spiking material and its preparation from the standpoint of relevance to blood infectivity.

Second, whether you feel there is any particular preference for TSE strain or animal model in these types of studies.

Whether immunoassays for PRP TSE -- well we would like for you to comment about the use of immunoassays for PRP TSE in the context of these clearance studies, as compared with bioassays for infectivity.

Lastly, identification of manufacturing processes that might alter TSE agent properties. We would like for you also to comment on that based on what you heard and perhaps other things that you know about the agents.

DR. TELLING: Thank you. So, before we get an

answer, let's discuss the question as it stands. I would like to make it open to the committee for discussion right now. Does anybody have any comments or additional questions?

DR. SALMAN: This is a point for a clarification. I thought we had discussed in the previous sessions what FDA has done with the risk assessment.

To my knowledge, the risk assessment led to the conclusion that there is almost like very low risk or negligible risk related to the factor VIII. Maybe somebody from FDA can clarify that.

DR. TELLING: Could somebody from FDA comment and clarify on that?

DR. EPSTEIN: Thank you very much. Actually, in the October 2005 TSE advisory committee meeting, we discussed the model that FDA would apply to estimating the vCJD risk from U.S. manufactured factor VIII.

We didn't actually show an output of that model. We do intend to bring to a forthcoming meeting of the committee the results of that assessment.

Generally speaking, looking at the input parameters, we think the risk will be lower than for products made in the United Kingdom, but we have not actually brought forward yet an output of the risk assessment for U.S. licensed factor VIII.

DR. SALMAN: Thank you for the clarification. I think now that you remind me, you are right. Would you think it is better to try to do the risk assessment before we decide about the protocol for how you assess the clearance of the plasma?

DR. EPSTEIN: I don't know that that is a question that just one person should answer. We do think that the risk assessment is highly informative.

The main message, though, is that the factors that most affect the assessed risk are the clearance, the prevalence in the donor pool, and the product usage in the patient community.

Far and away the largest variable really is the clearance. That is why we felt we need an antecedent discussion about just how well do we understand clearance and how concerned should we be about the absence of standardization in the data that has been brought forward to date.

I am not saying it is an absence of quality work. As Dr. Kreil pointed out, there are a lot of efforts made to assure high quality experimentation, but there is a lot of underlying variation. That is what we are trying to get at today.

I understand your point, that these things play back and forth against each other, and it is an open

question.

DR. SALMAN: One issue here is important. The first step in the risk assessment is hazard identification. As you stated in the beginning, the plasma source sold in the United States or marketed in the United States comes only from the United States.

To our knowledge, we don't have any native new variant CJD cases in the United States. Is that correct or not?

DR. EPSTEIN: That is correct. There are two cases, both of which are thought to have been acquired abroad.

However, as Mr. Cavanaugh correctly stated, there are persons who have had exposure, residency or travel, in parts of Europe where BSE has been prevalent and where vCJD has been reported.

Some of those persons may donate in the United States. For example, a person may have resided in the United Kingdom between 1980 and 1996 but for a period of less than three months. That person is not deferred as a plasma donor.

For instance, a person might have resided in France between 1980 and present but less than five years. That person might not be deferred as a plasma donor.

There are persons in the United States who have

had some level of exposure. However, the likelihood of donation by someone infected with vCJD as a U.S. plasma donor is certainly much lower than it is in Europe.

DR. LEITMAN: Could I follow up on that question which I think is very important, which I am having trouble grappling with?

In the United Kingdom hemophiliacs, one would think they would be at the very highest risk of having acquired variant CJD through the infusion of clotting concentrates, especially from 1980 to 1996.

Is it not correct to say that there has never been a single reported case, including post-mortem studies of brain, of subjects who died of other causes with hemophilia?

So, even in the best prospective surveillance system in the world, which is probably the United Kingdom, that has not been demonstrated; is that correct?

DR. TELLING: That was my understanding from this morning's comments, yes.

DR. LEITMAN: That has to be part of the hazard analysis. So, compared to that hazard in that population, I don't see how you could even make an assessment of hazard so small in U.S. recipients of clotting factors.

I have trouble with all of those questions because there are so many other more pressing issues to

address than the ones we are asked today.

DR. SCOTT: I would like to make some additional comments. The first is that, although it is true, as far as we know, nobody who has received a clotting factor or other plasma derived product has come down with this disease in the United Kingdom.

I think it is useful to remember that even in people who receive at least a whole unit of infected blood, it took them six and a half to eight years to come down with a clinical infection.

Now, you would imagine that the amount of material that they received through infected blood would be quite a bit greater than what they received through a plasma derivative, which perchance, would have some clearance during manufacturing.

Since these diseases have very prolonged incubation times, I think we can't really say for sure whether or not people have acquired this infection from clotting factors.

That is part of what drives our concern. Also, I want to comment that, although we don't have the results of the factor VIII risk assessment for you today, that what we are asking you really for is a scientific opinion about how we might think about and address these particular questions that are at hand, about how to do, if you will, the best or

the current optimal TSE clearance study.

We are not asking you to tell us exactly how to standardize it. We are asking you for your opinion about what you think, based on your scientific evaluation, might be useful for us to know and consider in looking at this.

DR. TELLING: Dr. Epstein, and then maybe we should go through the points in order.

DR. EPSTEIN: Dr. Scott has covered the main point that I wanted to make, which is that the absence of cases doesn't rule out long incubation periods in people who got exposed to a very low level of infectivity, and that is the source of the continued concern.

DR. TELLING: So, these concerns notwithstanding -- we have got one more question here.

DR. BROOKMEYER: I had a question about the risk assessment. The impact of errors on the estimated clearance rate on the risk assessment, if you are off by -- I am trying to get a sense of how accurate you need to get that clearance rate.

If you are off by, say, a factor of two on the clearance rate, not on a log scale, what would that do to the risk assessment, to your estimate of risk?

I don't know if someone from the FDA could comment on that in terms of is the error directly translated to the risk assessment.

DR. TELLING: Dr. Scott might be able to. It may be that at the next meeting, where we actually discuss the risk assessment model, it might be the more appropriate venue to do that, but if you do have some comments along those lines and if you would like to share them with us?

DR. SCOTT: I think we can't share numbers, but at the last meeting we discussed with the committee how many estimated logs of clearance or how many stratifications and ranges to have in the risk assessment analysis.

So, the choice was two to three logs clearance versus four to six versus seven to nine. You -- I expect you will be able to see that, but certainly a two-fold difference wouldn't have a great impact, but the sensitivity analysis, since it identified this as having major impact overall, the logs difference of clearance may be important.

DR. JOHNSON: Glenn, maybe you can clarify this. One of the issues that comes up, certainly in answering both number one and number four, is the effect of aggregation on infectivity, and how much more aggregation there is likely to be if it is prepared from brain, which is the only place where you could demonstrate those kinds of log changes, and a lot of these things are hinged. Do we have any good data on aggregation?

DR. TELLING: I think there was some evidence this morning presented suggesting that you can sediment infectivity but still recover significant amounts of infectivity, nonprotease resistant, in the supernatant. I think that may be addressing somewhat your concern, but I think beyond that I am not quite sure if there are any firm data on that issue.

DR. JOHNSON: But it really has to be -- you have to bioassay that infectivity.

DR. TELLING: That is correct.

DR. JOHNSON: I think if you show the western blot activity being one place, the other is not sufficient in my mind.

DR. TELLING: Weren't those data referring to infectivity assays? I am sorry, I am talking about the last statements that we heard, the last evidence, Dr. Kreil?

Can you discuss some evidence suggesting that you could sediment material and recover significant amounts of infectivity in the supernatant? I guess it would be equivalent to SPRP scrapie; is that correct? Was that by bioassay?

DR. KREIL: That was by bioassay. In that supernatant, the detection by western blot was attempted in two -- there were two attempts in detection by western

blot, one successful and one not successful.

So, there is very little PRP<sup>SC</sup>, the proteinase resistant form, but there is substantial ambiguity in that supernatant.

DR. TELLING: Thanks. I think maybe it would be prudent if we went through each of these points one by one for discussion. So, let me just rephrase it.

Point number one, what do you consider to be the optimal spiking material and its preparation from the standpoint of relevance to blood infectivity.

For example, SC 237 RML, would that be a good metric for understanding infectivity in blood of a vCJD patient, for example? Would a brain homogenate, as opposed to a spleen homogenate, for example, be a suitable surrogate?

DR. JOHNSON: Glenn, haven't you already defined that it is going to require brain by saying that you are going to drop it three to seven logs? The only place you are going to get those kinds of titers to shoot at is with brain, brain or spinal cord?

DR. TELLING: Any ideas of the titers of infectivity at earlier stages during infection in spleen, for example? You are right. If you are looking for a six log reduction, then brain would be the best.

DR. MANUELIDIS: I think 263K spleen is about

five logs less than brain, and I think in mouse models there is actually a higher infectivity in spleen in many of the CJD models, sporadic CJD, and especially the Japanese version of CJD.

So, they will actually be about 100 to 1,000-fold less than in brain, which is not bad, considering that for issues after one strain it is about  $10^9$  infectious units per gram.

DR. TELLING: So, you could approach, with certain strains and animal models, the levels of infectivity in the spleen, for example, that might be required to demonstrate a significant log reduction in infectivity, although brain is probably the better starting material.

DR. PRIOLA: Except that one of the considerations is that the physical form of infectivity in the brain is going to be significantly different than the blood.

So, this gets back to the aggregation bit or whatever. So, using lower titered material probably wouldn't get around that, because it is probably still in the same physical form.

What about the possibility of taking advantage of Dr. Safar's observation that PRP<sup>SC</sup> binds to LDLs and VLDLs, and possibly mixing those with brain homogenate, isolating

those -- if that is possible, i don't know anything about LDL biochemistry -- and then using that as a spike, because that is something that normally circulates through the blood. If you load it, then, with PRPSE, maybe that would be physically more representative. Maybe not.

DR. MASTRIANNI: I am grappling with the terminology, optimal spiking material versus what do we have available to do the job.

I mean, that is the question. What is the best form of scrapie to use here. Brain material probably isn't the best form.

I think as far as the LDL question goes, that is a good idea and a good possibility if it is biologically significant.

If we knew in vCJD patients, for example, that we could separate, get scrapie from the LDL fractions, then that would be a great starting material and, if it could be concentrated in that way, it would be probably the best starting material.

The bottom line comes down to the fact that we don't have vCJD patients' blood to be able to use as optimal spiking material.

So, I think we are left with thinking about brain for the question of the several logs of infectivity that we can have as proof positive that we are doing something in

the preparation, and the question is whether we can do some preparation that closely assimilates what might be seen in the blood, and specifically if we could characterize it or compare it with an actual infectious model, maybe that shows that scrapie is contained within the VLDLs. That would help.

MR. TELLING: I think probably the optimal spiking material might be, if we are going to use brain, vCJD brain. The problem is that the kinds of bioassays that would be required for sensitive detection of vCJD infectivity are not as well developed, with the exception perhaps of the bovinized transgenic mice, which appear to respond very well to variant CJD. So, that might be one avenue that we could explore in the future.

MS. MANUELIDIS: I think the other thing is, there is the R3 mouse model, which apparently also is fairly susceptible, within 300 days, to vCJD.

DR. TELLING: The R3 mouse?

DR. MANUELIDIS: Yes. That is even, I think, even across species. So, in fact, one might consider using, for instance, sheep blood where you can get a lot of it and then see if any of that is infectious. You have huge amounts of blood that you can collect from a sheep with BSE, et cetera. I just raise this as a possibility.

DR. TELLING: That probably falls under the

endogenous category rather than spiking, but yes.

DR. PRIOLA: I think you always have to go back to brain homogenous just to start, because it is what we probably understand the most about in terms of fractionating, infectivity, removing infectivity.

Another possibility -- and I think Dr. Kreil brought this up -- are this GPI minus transgenic mouse model that was created by Michael Oldstone and my boss, Chris Cheeseborough. They found lots of infectivity in the blood using at least one strain of mouse scrapie.

DR. TELLING:  $10^7$  ID 50s per ml.

DR. PRIOLA: Yes, something like that. If that is the case, whether or not it is in the correct physical form, I don't think we will resolve that question any time soon for any form of infectivity, but there you have got a form of infectivity in the blood that is transmissible.

That would be a spiking material that could be used with current rodent model systems. It wouldn't be a terribly difficult thing to do technically, I think. So, that is another possibility. Of course, it is not variant CJD or BSE related.

DR. TELLING: Spiking and endogenous model.

DR. PRIOLA: Exactly.

DR. TELLING: Any other points about optimal spiking?

MS. KRANITZ: This isn't about optimum spiking. As the consumer representative, I really don't qualify to delve into that. I can listen and semi-understand, but I did want to make a couple of comments.

We have family members here in this country who, in fact, two of them at present, one recently lost a loved one and another one is actively dying of CJD, both of whom were born in the United Kingdom and lived there the first, one 16 years, one 18 years.

So, we don't really know what we are dealing with in this country. Obviously, because the United States has cut back on their testing, their surveillance of animals, then the human surveillance should be increased.

I think it would be important to remember that we have an obligation to do everything we can to protect our American population, considering that this enigmatic disease still has a great deal of unknowns.

DR. TELLING: Thank you. So, I would like to move on to point number two, selection of TSE strain and animal model. I think that the two points are interrelated.

I think that probably we have addressed as much as we can concerns relating to materials, optimum materials, through use of spiking experiments.

Obviously, the models are in place, including hamster infectivity and mouse adapted scrapie infectivity,

and the appropriate host would be the relevant ones.

One could imagine that BSE adapted mouse infectivity, for example 301V, might be an appropriate TSE strain and the appropriate mouse model that would be more in line with certainly BSE and perhaps variant CJD. Any other comments along those lines?

DR. GESCHWIND: Just, I guess, to reiterate the point, the importance of using both human CJD and also possibly using humanized mice.

I will give the example of the chimera mouse Carston Court(?) has helped develop with the human mouse PRP. They were greatly able to shorten the incubation period with just a two amino acid substitution.

Unpublished but, with a third amino acid substitution, they are now even further able to reduce it down to, I believe, about 70 days.

Having a humanized model in human would probably be the best model for human infectivity. At a minimum, I think that should be included in any criteria.

DR. TELLING: Again, that was with sporadic CJD. Okay, let's move on to point number three, TSE immunoassays for PRP scrapie and bioassays for infectivity.

I would like to open this for discussion. Obviously, we have heard a lot about the CDI and its comparisons to bioassay.

The comparisons appear to be robust and reproducible, not just for CJD but also for chronic wasting disease and BSE; correct?

That would obviously be a very informative alternative to bioassay, and much more facile as an alternative.

DR. MANUELIDIS: I am making too many comments, but I do think that there are caveats you have to really be aware of between that.

I think one of the things that hasn't been done is, there are new tissue culture models, there are several models that offer the potential to look at infectivity in a much more rapid way.

I haven't seen anything sort of suggested on that or doing that, and I think that you are speaking about something that might take 30 days versus, let's say, 300 days.

DR. TELLING: I think we are going to hear from Dr. Soto tomorrow, but perhaps relevant to this discussion would be the new techniques that are emerging for sensitive and rapid detection of infectivity by in vitro amplification. Any thoughts about that or comments along those lines?

DR. PRIOLA: I would say that, with all of these techniques, they still have to be very, very carefully

validated against the bioassay.

I think that the PMCA, which is what you were just referring to, and the CDI show great promise, but really need to be carefully compared in the appropriate bioassay system at the highest level of sensitivity that we can get, if we are going to switch over to something that is sort of in vitro versus an in vitro diagnostic, especially for the low levels of infectivity that are in the blood.

DR. GESCHWIND: Just to follow up on that, I think that certainly for right now, I think one important thing is that we need both the bioassay and one of these other assays, that neither is going to be sufficient, even if we do find that the CDI or other assays are even more sensitive.

It probably would still be very important to show that you actually have transmissibility infectivity. So, I think both are going to be necessary.

Dr. TELLING: Okay, finally, identification of manufacturing processes that might alter TSE agent properties. We have heard something along these lines this morning from Dr. Kreil. Any other thoughts or comments about this?

DR. COLVIN: One thing I think we should keep in mind when we think about this part of it, too, is what the

numerator and the denominator of the problem are, which is obviously very difficult to sort out.

If we are thinking about factor eight and if we go back a decade or more -- probably more in this case -- in thinking about what the story was with HIV transmission in terms of how many donors there were in each lot of clotting factor that was used, initially the manufacturers would say things like, it was from 6,000 donors. Ultimately we learned that it was probably somewhere between 60,000 and 100,000 donors.

Now, if you take the UK sample that Dr. Scott talked about earlier today, that there were three young people who died or had tissue samples of unrelated causes out of 12,000.

If we are talking about a denominator of what is in the clotting factor of 60,000 donors, then we would be thinking about 15 or so people who would have potentially asymptomatic infection.

Now, we don't know if that is going to be a contagious infection or contagious material, but I think we still need to think that there may be some level in there that is higher than we actually are anticipating, or people who potentially can transmit this disease.

DR. HAMILTON: I kind of have a follow up to what Dr. Colvin was saying. Is there any data at all that

specifies -- we know it can take up to 38-1/2 years to show up, but is there any data at all that specifies how far from actual symptomatology that the donor is contagious and could transfer that through a donation? Is there anything at all that shows -- I mean, three years, five years, 10 years? Do we know at all?

DR. TELLING: In terms of animal models, what we heard from Dr. Brown last time was that halfway through the incubation period to the end, to the manifestation of clinical symptoms, was the time at which titer could be detected in blood. If anybody has any better recollection of that than me?

DR. CERVENAKOVA: My name is Larisa Cervenakova. I am from the American Red Cross and I just would like to comment on these animal studies, which actually showed that using the mouse model for the strain which is a human derived strain and for variant CJD strain and for scrapie 263 strain in hamsters, it seems that through half of the incubation period you have the infectivity present in plasma and in the animals.

The pattern goes up to the clinical stage of the disease, but there are some discrepancies in studies which were performed by Claudio Soto's group when they compared the infectivity in the blood of hamsters, and as they tried to correlate it with the data of PMCA. It seems that, at a

certain point close to the clinical stage of the disease, they were not able to find the presence of a normal PRP in plasma of the animals, and they were able again to detect it at the clinical stage.

DR. HAMILTON: So, in other words, that could be maybe why some of it is not showing up in UK hemophilia patients? It just hasn't been long enough?

DR. TELLING: I think the comments of Dr. Scott would certainly underscore that.

DR. COLVIN: Something else that is relevant to this part of the question, I am wondering, given the new data that we heard from Dr. Safar today, or relatively new data, where does LDL and VLDL, as opposed to some other things, fractionated in the fractionation of plasma, does it fractionate with a cryoprecipitate or does it go somewhere else?

I think that would be kind of an interesting question. I don't think we are ready to say, well, this means that we are not going to see the TSE agents co-precipitate with factor VIII. On the other hand, it is something that I think would be interesting to look into at this point.

DR. LEITMAN: It is not cryoprecipitable.

DR. COLVIN: VLDL and LDL.

DR. LEITMAN: No, very few things are

cryoprecipitable. Maybe someone from PPTA could confirm that, but the two to four million dalton cholesterol moieties are not cryoprecipitable.

DR. KREIL: Really, obviously in preparation for that meeting, knowing that Dr. Safar was going to share that newly recognized association between VLDL and prions, we were trying to find information where it would fractionate into.

The best information that we found was actually from the early days of the Cohn fractionation. It seems that we are going to fraction number three. That is currently not being used for manufacture of further products. So, that would be a waste fraction and therefore would be good news.

Again, this is very preliminary information, certainly. It is something that we would want to take a look at.

DR. SAFAR: I think that is exactly the issue we are looking into, where specifically the sCJD prions are. There are so many fractions with spectrum of lipoproteins. So, the class that we identified is pretty broad itself and it has sedimentation properties that are in the still very broad range.

How each of those classes will behave in any industrial production protocols or procedures, i don't

think there are sufficient data to even guess at this point.

I would agree with the comment that it probably would go to the fraction three. There are additional aspects.

There are specific protocols, plasmapheresis or lipophoresis protocols for removal of LDL from plasma, using specific sized filters, which seems to be working for clinical cases with hypercholesterolemia.

So, there is some technology and some information for it, but it is not very precise, and it is definitely unrelated to the CJD issue.

DR. TELLING: I think we will move on. There is a question here.

DR. HOGAN: Before you move on to question 1-B, I just wanted to comment, the very beginning of this in 1-A it asks about the feasibility and scientific value of adopting standardized procedures.

I think we have heard from all these people that we really don't know what the best spiking agent is, we don't know the best model, we don't know how we are going to assess.

I think the idea of adopting at this point would be a little premature. Certainly this is all discussion for some time in the future, but I don't see anything we

can adopt right now.

DR. SALTER: Just to follow up on that point, we heard from Dr. Kreil that the manufacturing processes are not standardized.

So, developing a standard for a clearance study may not accurately reflect the flexibility that is needed, just due to the processing itself. So, that is something that I think we need to remember.

DR. SCOTT: I want to thank you for these comments because we do recognize how difficult it is to try to simulate blood borne infectivity in a high enough titer to do clearance studies that would appear to have meaningful levels of clearance. It is also a fast moving science and we appreciate your input.

We did want to ask one additional question, if anyone on the committee would like to comment, just related to the first bullet that we had.

That is, does the committee see any reason for a preference for using brain homogenate as opposed to microsomal fraction or other fractions as a spiking agent.

DR. MANUELIDIS: Actually, I have a preference for using a brain fraction. I think brain homogenate is so incredibly complex that I think the most simplified fraction you have, even though it is not perfect, I think it is something that is more likely to partition correctly

in blood when it goes into blood or into plasma.

The more garbage you have in it, I think, the worse it is. There are many types of fractions -- noctosomal, microsomal, et cetera, non-membrane fractions. I think those really are preferable in many ways to a total brain homogenate.

I think also the kinds of conclusions that people draw, it is very nice to have a western blot of brain homogenate and say, well, this is PRP, but there are hundreds of things in that.

I think that sometimes one can be misled to think that one is only dealing with changes in PRP where, in fact, many things are being changed by the treatments.

DR. COLVIN: One thing I am not so sure about is this idea that if we have some kind of a standard assay or a standard number of logs we must reduce infectivity by, that that will stifle science.

I don't think that is ever the case. Any time in science we do something, if somebody comes up with something better, we are going to adopt that as well.

I just think at some point we need to say, well, do we want to say four logs is adequate, five logs. Can we put a number on it? The question is, what would be the spike that we would do it with.

I agree, it seems like the products are

manufactured differently with different procedures and the like. However, the idea, though, that because we have different products that means we can't come up with some idea of what -- we just ignore the whole problem entirely seems a little bit outlandish, in some way, to me.

I do think that, again, as soon as somebody comes up with a good assay we are going to adopt it and people are not going to stop trying to develop assays just because there is a standard.

We spend our time -- most of the time I spend my time as an HIV person. For example, developing assays to look at the effect of CCR5 antagonist, a new class of HIV drugs, sure we are trying very hard to find standardized assays, knowing that it is not going to take long for a better assay to be developed, but we want to have a standard now that we can not just wait for even the best assay.

I don't think we are -- we are never going to have the best assay. That is the nature of science. We keep moving ahead and make things better.

As of September 2006, I feel like we should come up with something that would say, well, we would like to say that we are going to get a four log reduction and we are going to do it this way but, as soon as somebody comes up with something better, we will use that one instead.

DR. TELLING: I think we can probably address that today, then. I think we can move on to question B, and we are being asked to comment on the feasibility and scientific value of adopting standardized endogenous study methods to assess TSE clearance in plasma derived factor VIII.

There have been some recent developments along these lines which have relevance. We have heard mention of a transgenic model in which infectivity in blood is substantially higher, orders of magnitude higher, which may hold some promise as an endogenous model.

Obviously sheep transfusion has been another recent development that lends promise. It seems to me also that -- this is obviously a personal preference -- possibly the use of infected cervids, DUNL, for example, infected with chronic wasting disease, because of the similarities between variant CJD, or at least some similarities between variant CJD and CWD, that this might also be an endogenous infectivity model that would be worth exploring. So, are there any other comments from the committee members about question 1-B, endogenous models?

DR. PRIOLA: Can you remind me, has anyone looked at mice infected with variant CJD for blood infectivity? Has Larisa or someone done that with mouse models of variant CJD or you?

DR. TELLING: I can't comment on that.

DR. CERVENAKOVA: The only study which was done, it was a study using focuoca(?) strain. These were done together with Baxter.

I don't remember the numbers. There was still some infectivity present in cryoprecipitate. I am really not prepared with those numbers.

We didn't look through the clearance steps. We didn't evaluate clearance using monoclonal antibodies with endogenous blood, which is a very important step in the clearance procedure.

This was done with spiked material. Thomas may add something to that. I don't know if they did some additional studies using endogenous blood.

Until cryoprecipitation was done with the endogenous blood and these particular steps, like depth filtration and monoclonal antibody were done with spiked material.

DR. PRIOLA: So, there really aren't any models of endogenous variant. The hamster model is the only one that has been relatively well characterized.

DR. TELLING: Well, the closest might be BSE in sheep.

DR. PRIOLA: But that is very expensive to do. That is not practical.

DR. MANUELIDIS: 301B in mice.

DR. PRIOLA: Is there infectivity?

DR. MANUELIDIS: I don't know. It is not from our group. I think it is the English group did it.

DR. CERVENAKOVA: 301B, it was done by Debbie Taylor(?). She found infectivity in the plasma of these mice, which was at the range which was established for variant CJD mouse model, which we have in the lab.

Nothing more than that was done for these particular models. If you talk about factor VIII, an endogenous experiment was done with fucuoca strain in the mouse model up to cryoprecipitate.

DR. MASTRIANNI: Were any of these models done by feeding or by injections?

DR. CERVENAKOVA: No, everything was done where animals were inoculated with intracerebral inoculation and blood was taken at the clinical stage of the disease, and was processed that way.

DR. MASTRIANNI: It would seem that a better model would be feeding the agent to an animal.

DR. TELLING: The animal model that probably comes high on the list in terms of scientific value would be a primate model of variant CJD, but this would have a lot feasibility, I think, at the moment. These models are available, but certainly not widely available. Has that

been addressed at all?

DR. CERVENAKOVA: Yes, it is too bad that Phil Brown is not here for these discussions. As you may remember, on some of the advisory committees data were presented on Baxter's experiment which was done using squirrel monkeys.

It was a significant study actually done in a way that squirrel monkeys were -- it was two phases, I believe. I am trying to recall some of the results slides.

Some of the animals were inoculated with sporadic CJD and variant CJD. Blood from these animals was taken every three months during their lifetime, and this blood was transfused into two squirrel monkeys. They always received this blood from animals which were developing this disease.

The study was terminated, I believe, five years after these transfusions. It means that during five years these animals got multiple transfusions of blood from squirrel monkeys which were inoculated with variant CJD or sporadic CJD.

None of the animals developed the disease, but their organs, I believe are still under evaluation by the CJD surveillance unit.

The other part of the study was that blood from chimpanzees, which were infected with sporadic CJD, was

taken and separated into components, and these components were inoculated into squirrel monkeys.

There was one transfusion from such a chimpanzee which developed sporadic CJD. There was another part, when blood from sporadic CJD patients and from variant CJD patients was separated into components and inoculated into squirrel monkeys. I believe there were also no transmissions.

DR. TELLING: Thank you. Any other information or comments?

DR. GESCHWIND: Just a comment and maybe you can comment on this as well. I guess one concern I have about using any single animal model is the differences.

One nice thing about using a cervid model is the size and they are captive herds, but the transmission among the cervids seems to be very different from what occurs in BSE as far as, for instance, the horizontal transmission, all the herd getting infected where with BSE, as I understand, it seems that the transmission seems to be from a single source.

So, that is suggesting that there are great differences in the way that these diseases are transmitted, and even with cervids we are not sure about how the transmission occurs. Is it through urine, is it through saliva, feces. Is it through something else?

I just think we have to keep that in mind, that each species is different, and that one method probably won't be sufficient.

DR. TELLING: Just to comment once again, the difference in the BSE in the natural host, in bovids, is radically different from its behavior in human beings. So, strain host combinations are extremely important.

DR. PRIOLA: Just very quickly, I remember from the last meeting this issue of multiple animal models came up.

I think the committee decided that there should be multiple animal models used where possible, to test these things, to take into account the differences.

DR. TELLING: We did actually go into that, didn't we. Okay, I am going to move on to question two. I will just rephrase it. Based on the available scientific knowledge, please would the committee discuss whether a minimum BSE agent reduction factor, demonstrated using an exogenous spiking model and scaled down manufacturing experiments might reasonably serve as an appropriate standard for demonstrating TSE safety of the products. So, are we comfortable with a four log reduction or a six log reduction? Does Dr. Epstein have something to say along these lines?

DR. EPSTEIN: I wanted to ask the committee a

question based on the endogenous experiments, and maybe give people time to think about question two.

Is there a feasibility to process a large volume of blood from a large animal, sheep, goat, cattle, perform the process but then attempt to concentrate the infectivity, so that you could do read out in a rodent model.

The basic barrier in doing the experiment entirely in the rodent is that you need thousands of rodents to look for any residual infectivity.

You could process very large volumes of plasma or whole blood from a large animal, if you could do the read out in a small animal.

I just wonder if the committee members could comment on the feasibility of available technologies to make concentrates of residual infectivity from whatever end product one made.

In other words, if you made factor VIII, could you concentrate the infectivity out of it and then read it out in a rodent? Then these kinds of experiments would be much more feasible.

Dr. TELLING: Not being an expert in blood concentration, but certainly having some expertise in the animal models, I think what you are proposing would be the way that I would want to do the experiment.

I would say it is extremely feasible and extremely informative scientifically with the currently available, sensitive and relatively rapid transgenic model for human, ovine, bovine at UCSF and various other groups in the United Kingdom and various other locations in the United States and Europe. I think those sorts of studies could very readily be done and would be very informative.

DR. MANUELIDIS: I also agree, that is very nice theoretically. Correct me if I am wrong. If you take a species like human, I mean, you could actually concentrate human blood or bovine.

When you go across species, even to the transgenic models -- and I have seen that with the BSE, I have looked at the BSE and the vCJD models -- in fact, it takes 300 days. That is with a huge amount of material intracerebrally, where you are going from brain to brain.

So, I really wonder if this -- although in principle I agree with you, I really wonder, with the current models that we have, whether it will work.

DR. TELLING: Well, incubation times range depending on the model and the host that the transgene is expressed, the level of expression, there are a variety of different factors.

They range from anywhere as rapidly as 30 days as, you are right, 300 days. Certainly these various models

are being continuously improved.

DR. MANUELIDIS: I would like to know what the 30-day model is. Would you get across species?

DR. TELLING: 30-day incubation time of SC237 in over-expressing transgenic mice.

DR. MANUELIDIS: No, I am talking about across species from the natural host. So, you take cow, bovine --

DR. TELLING: This would be hamster to mouse.

DR. MANUELIDIS: Bovine serum, sheep serum, that is infected with cVJD, a cow brain, whatever you want, and you go into a mouse, any mouse.

All the stuff that I have seen in the literature is basically 250 to 300 days even with the transgenic increased susceptibility.

DR. TELLING: For the humanized model, the most rapid incubation times that I have seen run about 100 days for the chimeric mouse human transgene.

Another alternative would be -- this is newly derived information -- that the Banglo looks to be a very promising host for a variety of different models of infection, scrapie, CWD, not so much BSE.

DR. SAFAR: The BSE in bovine transgenics, it is between 190 and 220 days for BSE intracerebrally. The issue in this case is significant and I agree with Laura in this case.

For example, BSE in R3 mice titrates into an end point only to  $10^3$  infectious units per gram. If you do the same experiment in bovine transgenics, you will titrate  $10^7$  or  $10^8$  infectious units per gram from the same material.

So, the species barrier definitely exists and it has to be considered in those experiments. So, I think there has to be a list of I would call it relevancies.

First, the prion species or strain relevant for that specific study, the clearance, decontamination, whatever.

Second, selection of the best host to do that study. Third, because a lot of assays are based on the immunodetection, it has to be also the sequence of PRP which is relevant for those assays which are going to be used in the detection of PRP, if the PRP is going to be used as a surrogate marker for infectivity.

In that case, every such assay has to be correlated, as the biologists say, to prove in principle that that link, that extrapolation from PRP<sup>SC</sup> levels that are infected, that it can be done.

DR. KREIL: If I can offer another comment, beyond the difficulties in read out, I think we should also keep in mind that it is rather better understood that, for example, mouse plasma fractionates totally different from human plasma.

I am not sure we know anything about the fractionation of sheep plasma. So, even if we used that as a source of endogenous infectivity and ran it through our manufacturing processes, I don't think that would generate any meaningful information for understanding of how that would translate into the fractionation of human plasma.

DR. TELLING: So, how do you feel about the spiking experiments, then?

DR. KREIL: Well, at least there we know that the process performs like it does in the large scale, which is why I pointed out that the validation of the down scale is so important, because otherwise you don't really know whether you generate information meaningful for the large scale manufacturing process.

DR. CERVENAKOVA: I would like to make a comment on animal models, and actually no one tried to do that, to use human plasma from sporadic CJD case and tried to put it through the procedure, and inoculate it into transgenic mice.

To my knowledge there are very limited studies done in transgenic mice in general using human plasma. I don't remember how many animals were inoculated at UCSF with human plasma from sporadic CJD cases, but it was just six or eight animals inoculated, R3 strain, inoculated with variant CJD.

From this number of animals you definitely may not get any results. In my experience if you take a group of five animals and a group of 20 animals and you inoculate those five animals and let them sit and you inoculate 20 animals and let them sit as a separate group, you will not see any infections in the group of five animals.

I believe that, if infectivity is present in the blood of variant CJD patients and there are some indications at least that abnormal PRP might be present there, we should be able, by concentration steps, to concentrate the infectivity and, by inoculation of transgenic mice, which carry human PRP gene and have a short incubation period, we would be able to see it, if it is there.

With variant CJD, it is a very different matter because, even so, even humanized transgenic mice, they do show sensitivity to the variant CJD, but the incubation period is so long, that we are not able to see low levels of infectivity which are present in blood.

This is true for bovinized mice, I call them, because they also will have a very long incubation period and we will be not able to see the infection if it is present in very low titer.

DR. TELLING: So, we are lacking a host readout for variant CJD, even if we could fractionate from variant

CJD infected blood.

DR. CERVENAKOVA: I believe so. We need to look for a good model for variant CJD for low levels of infectivity. I also am wondering -- I know that no one did it, actually, and we are planning to do the studies to try to use transgenic mice and blood from transgenic mice to prove if we can transmit the disease by blood, inoculate transgenic animals in between.

DR. GAYLOR: On question two, I think it is rather difficult to answer question two at this time without having the FDA risk assessment in front of us.

I had the advantage of reviewing the factor VIII risk assessment as of about a month ago. I don't know how much it has changed in the past month.

It is true that the most uncertain part of that estimation is the manufacturing reduction factor. That is the largest source of uncertainty.

You can assume various log reduction factors and estimate risk, but those assume factors. It would appear with, say, a log 6 reduction that I guess the best term to use is you come up with a negligible risk without defining negligible mathematically.

If the risk is -- if the reduction factor is only, say, log 3, you still may come up with a negligible risk. So, it is kind of hard to say whether log 3 or log 6

is what is needed. A log 3 reduction may be adequate. I don't think we can really answer that until we have the benefit of looking at the risk assessment at the next meeting.

DR. TELLING: Could we defer this question until that time? I think we will do that then. I am sorry, there is another comment here.

DR. POWELL: It may be the committee's decision to defer the question of the specific value, but in terms of some comments that FDA might consider as they think about that, I think one thing that is important to consider is how a log reduction is defined.

The nominal log reductions that I have heard talked about I presume to be average values and average log reduction achieved in one or more replicates.

DR. SCOTT: That is correct. So, you have a starting absolute amount of infectivity, which is the volume of material that you have times the amount of infectivity per ml.

Then you have your manufacturing process. You will probably come out with different volumes at the other end. So, you calculate that absolute amount of infectivity and you subtract it from your starting material.

DR. POWELL: When we speak about a four log reduction factor, I think it is important to consider not

only the nominal value or the mean value, but also whether there is some variability in the performance that you might detect through replicates under the same conditions.

For example, you might have a process -- two processes -- that both achieve an average log reduction of whatever -- four -- but the one that has the greater variance in terms of its performance is going to result in a greater likelihood of high levels of residual contamination.

So, it is not simply the average performance but also the variability in performance that is important to consider when you are formulating a performance standard for a log reduction of whatever process.

For example, it might be formulated as 95 percent confidence of no less than X log reduction as opposed to a more ambiguous nominal log reduction.

DR. SCOTT: That is exactly correct. Because each manufacturing run has slight differences in reality in terms of the exact amount of alcohol, the precise pH, there is some variation, especially when you are talking about precipitation studies, which are not generally considered, if you will, robust.

In viral validation studies, it is possible -- because you can have a fairly high throughput of number of experiments -- to look at robustness and see how much the

variations in the manufacturing ranges can impact that, and come up with a plus or minus this and a confidence interval.

That is a lot more difficult in these studies if you are depending upon assays like bioassays that take a long period of time.

It is not impossible, but you are absolutely right. There is a lot of information in the literature of replicate studies.

In general, it would be possible, I think, for us to come up with how much range there is in some of these studies as published that is useful.

DR. BROOKMEYER: This question is posed as if there is a single reduction factor to be aiming for. The reduction factor could also depend on what the input is that you start with.

If you start with a lot you might have a big reduction, where if you start with very low amounts, you may not have much of a reduction factor.

So, there is going to be some swing here in the reduction factor depending upon what your baseline is and what you are starting with.

My question is, is that right, and is there any data to reflect on that, on how the reduction factor could vary with what your initial input is?

Wouldn't that be a critical question, actually, for the risk assessment and for assessing your absolute risk here?

DR. POWELL: You are speaking about a concentration dependent effect of the performance of a process as opposed to an incoming load variant, specifically the concentration dependency of the process in terms of its efficacy.

DR. BROOKMEYER: That was the question I had, too, in looking at the literature, because we see this in antibody antigen dynamics, that there is a dependency. I am wondering, in some of these precipitation sorts of processes, there might be a similar sort of effect.

DR. MASTRIANNI: I had exactly that same question. Along the same lines as Dr. Kreil was speaking about, log reduction factors, he was adding them up and I wonder if that is a reasonable thing to do also. Maybe you can comment on that. When you are adding logs, you are actually multiplying factors; right?

DR. KREIL: Well, obviously that is something that needs to be demonstrated and I guess the agency's terminology has been where steps can be demonstrated to be orthogonal, so different in terms of mechanism of action, then it is appropriate to add them up. If that cannot be demonstrated, then it would not be.

DR. MASTRIANNI: Do you have any data to suggest that if you start with a higher titer of infectivity the log reduction is different than if you started with a lower titer of infectivity?

DR. KREIL: I would not be aware of nay such data. That would assume that the mechanism would be a saturable mechanism, where the partitioning is not.

DR. PIFAT: I had a comment. I am from Telecris. We have conducted experiments where we spiked plasma and carried the manufacturing process almost to completion and have shown clearance over the entire process, and have been able to show that actually small levels of clearance, one or two logs, that are contributed by individual steps actually add up when you conduct the experiment from start to finish.

DR. SCOTT: It is the empirical way to determine whether steps are additive or not, and it would seem the most valid way to do it is actually to do the steps both separately and in sequence.

There are some published examples where those either have or have not been additive. It is a little hard to predict what is going to happen without doing the experiment.

DR. POWELL: One other issue regarding things that FDA might consider in terms of a performance standard,

is that we are dealing with a continuous unit as opposed to a discrete unit.

Typically, in microbial risk analysis when we think about a log reduction, say, down to a mean dose of -- well, let's say we start with four logs and apply a four log reduction factor. We have a mean dose of one discrete unit per dose, because that is a discrete, countable colony-forming unit or what have you.

You have -- if you assume, say, a puissant process, there would be, oh, probably about a 40 percent probability that any particular dose that you selected from that lot would be absolutely free of any infectivity.

That is not the case with an ID50, which is a continuous variable. So, applying the sorts of procedures for your highest reasonable concentration plus a two log safety factor is not, I think, directly relevant when you are dealing with a continuous unit like an ID 50, as opposed to a discrete unit, like a colony forming unit in microbial risk analysis.

DR. GESCHWIND: Maybe this is more of a question, but sort of the way I think of this is not so much log reduction, but we would want to say that you have reduced the infectivity to below one infectious dose. That would seem to be it. I am wondering how that fits with what you are saying.

DR. POWELL: Again, your unit is not one percentile. It is an ID 50. It is the median infectious dose.

So, one ID unit is actually the dose that would make 50 percent become infected. So, the unit is quite different than the countable units that we typically deal with when we are dealing with virus or infectious bacteria.

DR. GESCHWIND: So, just to follow up with that, then, is there a solution to that? I think that is the reality of what we are dealing with. Are we going to have to get to the point where there is never an animal that gets infected, which would be zero ID?

DR. POWELL: It would be a function of your desired level of protection. Simply from the calculation perspective, it would be inappropriate to use the sort of procedure that you would use for bacteria and apply it to an ID 50, which is a continuous unit.

DR. MANUELIDIS: I actually have a question about that. In virology, as I understand it, most of the viruses are usually multiple.

In other words, even if you do a colony count, there is a certain efficiency. Maybe the one exception might be polio virus, which can have a one to one article to, let's say, colony change.

Virtually most of the other viruses are like 100

to one or something in that kind of range. Maybe you want to sort of clarify how you would -- I think it is fair enough -- one ID 50 is essentially saying, well, you want to be something like at least 10-fold under that, I think, if you want to say it is clearance.

I assume that the infectivity, particle infectivity, is relatively low in these diseases, as it takes a long time to get, but I may be totally wrong, but I would say something 10-fold.

DR. POWELL: Similarly, with bacteria, we can count colonies. We don't know how many bacteria are members of a particular colony, but this is more akin to -- an ID 50 would be more akin to -- an ambient concentration of a chemical pollutant.

Obviously, at some level we are getting down to a fraction of an ID 50 that constitutes a countable infectious unit.

The idea that the two log safety factor that is kind of a convention in microbial risk analysis could be directly translated over to dealing with ID 50s, I think that FDA needs to consider that.

DR. TELLING: Well, I think these comments are probably very useful to FDA. We will move on to question three, which is somewhat related.

So, the question is, considering the outcome of

discussions on question two, notwithstanding the fact that we are going to just defer this, in cases where only lower levels of clearance can be demonstrated for a plasma derived factor VIII, should FDA consider the following:

A, labeling that would differentiate the lower clearance products from other products with sufficient TSE clearance; B, recommending addition of TSE clearance steps to the manufacturing method; C, performance of TSE clearance experiments using endogenous infectivity models; D, any other actions.

DR. GAYLOR: Again, since three depends on two and we have deferred two, it is a little hard to -- we could still discuss three a bit, but it is kind of hard to come to any conclusions on three without concluding on two.

DR. TELLING: I wonder if the committee had any thoughts relevant to this that would be useful for FDA to hear.

DR. LEITMAN: I am a little concerned about A. Labeling is something that we do for the public. So, labeling something as differentiating it as being less good than something else is a concern.

It raises anxiety on behalf of the public. We are talking about negligible risk to begin with right now. I don't think any labeling should be discussed at this point.

DR. JOHNSON: I was also going to question about

A. Is this considering a required labeling saying, in the standard tests, this material is better than X,Y,Z but not as good as P,Q, or is it allowing labeling, permission to label? What is FDA's position on that?

DR. TELLING: FDA?

DR. GESCHWIND: Maybe toward this, if FDA could also comment about products that have already been labeled that were alluded to in the handout?

DR. EPSTEIN: Our current policy is that we have permitted voluntary labeling of clearance when we think we have seen data that are robust and high quality.

We have not allowed those labels to further assert any greater assurance of safety, only to assure the public that there are these clearance experiments and here is what they show.

I think at the present time we are not talking about mandatory labeling, but if we thought it was a safety issue, then potentially it could be mandatory.

Right now, what we are talking about is labeling related to clearance with some uncertainty whether that is linked to a level of safety assurance.

The point of that, why we have allowed that at all, is that we wanted to provide an incentive for companies to do the kinds of experiments that you have been hearing about. So, that is not a clean answer, Dr.

Johnson.

DR. GESCHWIND: I just think that it is important, related to that, that the market place is going to drive the better product.

If there are tests or if there are methodologies out there that do provide higher clearance, that is something that consumers might want, of course albeit with the caveat that clearance may not be infectivity. That is what Dr. Epstein alluded to.

DR. TELLING: I am sorry, what was the last?

DR. GESCHWIND: That just because you have shown clearance doesn't mean that it is necessarily a safer product.

MR. BIAS: After this decision is made, most of us will go home and life won't be any different. If you are a patient who infuses his product, every company that has that advantage of removing that many more logs -- I remind the committee that we haven't seen any infections related to plasma based products in many, many years -- those are the people who are going to get that message.

It is going to show up at their door. It is going to show up from the people who deliver their products. It is going to show up at every hemophilia meeting they go to.

It is going to be used as a strong marketing tool to separate one product from another, and there are some

complications there.

Some of these products are true plasma products. Some of them have von Willebrand factor in them, which is essential to patients. We are finding more and more patients who have hemophilia and some sort of von Willebrand factor missing and need products that have both strong hemophilia and some von Willebrand factor in them.

It is a much more complicated issue than allowing the manufacturers to claim an advantage through labeling that really turns into a strong marketing pull.

We have, among the recombinant manufacturers, we are finding in local communities now that they are pulling together small groups of consumers in private situations without physicians present, and pitching their products as if one is substantially better than the other.

Well, we have seen no infectivity or transmission in any of the products, no differences whatever. It takes the decision out of the physician and the patient's hands, and makes it into a marketing tool for the manufacturer.

So, at this time, I couldn't support any labeling change based on lower clearance, because the products are so unique to the patient's needs. It just seems inappropriate at this time with so many unknowns, in my opinion.

DR. SEJVAR: I guess just to build on that, if

the labeling is for the benefit of the consuming public, I am not so sure how much of a service there is to make these statements in the absence of any clear idea of what the biological relevance is. I guess I feel a bit hesitant about the whole labeling issue as well.

DR. MASTRIANNI: I guess one could imagine just a standard label that says, meets criteria for FDA requirements of being prion free or something to that effect, that all things are labeled with a certain standard to suggest that either risk from infection is very low based on the product and/or has met some criteria which do come from this meeting as far as standard levels of reduction of infectivity.

In other words, just normalize it or equalize it for all the different companies, just create a standard rather than differences in higher or lower levels of standard.

DR. TELLING: Let me ask if FDA is satisfied with the comments that have been presented to them so far. I think that while there is some uncertainty -- a lot of uncertainty -- there has been some constructive discussion here. Would that be fair to say?

DR. SCOTT: It has been very useful, but if committee members have comments on B or C, and let's just take a hypothetical that, at some point, the committee or

we felt that there wasn't a sufficient level of clearance, what do you think about options B and C, and are there any other actions to consider in that scenario?

DR. PRIOLA: With regard to C, thinking about what Dr. Epstein had asked about large animal models or in concentrating, and what Dr. Kreil said about the fact that blood from these models does not fractionate in the same way as human blood, it would seem that, while it is feasible to use some of these rodent models of endogenous infectivity, the scientific value might be limited, given that they don't fractionate in the same way as human blood.

So, it might be that performance to TSE clearance experiments using endogenous infectivity models, as in C and I guess 1B, might not be so feasible scientifically if they don't mimic the way human blood fractionates.

DR. HAMILTON: Regarding B, I think we are always recommending -- and manufacturers know that -- the committee is always recommending do whatever you can to make the product safer and as safe as possible.

I would have to agree with what Mr. Bias said, to some extent. Unless a product is really, really, really better in this line, that we be very careful about what we say about the products without enough substantiation for that.

Like you said, it is very difficult to convey to

the consumer, I think, adequately without fear of what the risk is.

Clearly, from the discussion today, we don't really know yet what the risk is and, without being able to explain that to the patients in a way that they can understand, I would be very leery of any current extra labeling.

DR. TELLING: So, I think we would like some more detailed and thorough assessment of the risk, maybe in the next meeting. Are there any other comments? Dr. Scott, before we adjourn?

DR. SCOTT: If there aren't any other comments, I would like to thank the committee and assure them that we are planning to present the risk assessment at some future date, and we appreciate all your thoughts that have gone into these questions. You will probably be hearing some version of them again.

DR. TELLING: Okay, thank you very much. Let's adjourn for a 15-minute break and we will reconvene at just after 2:45. Thank you.

[Brief recess.]

DR. TELLING: So, we are going to get back to committee updates. Dr. Weinstein, from the FDA, is going to update us on the status of FDA's initiative on communication of the potential exposure to vCJD risk, and

an investigational product, plasma-derived factor XI that was manufactured from United Kingdom donor plasma.

**Agenda Item: Committee Updates: Status of FDA Initiation on Communication of Potential Exposure.**

DR. WEINSTEIN: As many of you know, there is a possible but as yet unproven risk to approximately 50 individuals who were involved in investigational study of a product, a factor XI product, that is used to prevent or treat bleeding in these patients who are deficient in factor XI.

The factor XI was made using plasma from donors in the United Kingdom where the human disease variant CJD has occurred.

Now, it is important to note that the factor XI product was not made from the plasma of anyone known to have developed the disease, and that no one who has received this product is known to have become infected from it.

Now, although the product was not made from a plasma from anyone who developed variant CJD, it is still possible that someone using the factor XI product could have been exposed to the agent because of a donor who felt well at the time of blood donation, who was actually carrying the variant CJD agent.

Now, FDA has made a computer model of a risk

assessment, and we have presented this risk assessment to you in open session in February 2005, and we also received comments about it and information when we were discussing the factor VIII model in October of 2005. We have taken those comments and have revised the risk assessment.

One of the important elements of your recommendations was to advise the FDA to consult with SGEs -- that is special government employees -- including members of the hemophilia community, to obtain advice on risk assessment, on interpretation of the assessment and on its communication.

Again, by way of background, the factor XI was manufactured in the United Kingdom from UK plasma, and was used by approximately 50 patients between 1989 and 2000.

Most of the cases of variant CJD have occurred in the United Kingdom. We heard today that 162 out of 196 reported cases have occurred in the United Kingdom.

Individuals in the United Kingdom are at higher risk for the disease than individuals elsewhere because of the greater potential for food chain exposure.

In the United Kingdom, health authorities have notified recipients of plasma derived coagulation products, which were of course made from UK donor plasma, that they might have an increased risk of variant CJD in addition to the risk of eating contaminated beef products.

In CBER's risk assessment model, which again you have had an opportunity to comment upon, we found that the most important factors affecting the risk are the clearance of the variant CJD agents through manufacturing, how much of the product individuals use, and the prevalence of variant CJD in the United Kingdom donor population.

Now, we have completed the risk assessment and we have distributed it to SGEs, to the hemophilia community. We are waiting for their comments, which we anticipate getting very soon and, once we have those comments, FDA will present the risk assessment, an interpretive summary of the risk assessment, and advice to patients.

We intent to submit this risk assessment and discuss it with the IND holders of the factor XI products to obtain their comments and to answer questions about the risk assessment.

We will strongly suggest that the IND holders contact and advise patients, the subjects who participated in this study, and there will be a public posting of the risk assessment and notification to hemophilia organizations of the risk information.

We will have a follow up among FDA and IND holders to assess the impact of the message, the number of patients contacted, and the need for additional information or assistance from the FDA, and for the Centers for Disease

Control and Prevention. Thank you.

DR. TELLING: Thank you, Dr. Weinstein. Are there any questions or comments?

MR. BIAS: I am curious. I notice the different points of communication here and that is pretty good, but from that list, I don't see any communication to physicians.

The way hemophilia is treated, as you know, is through a network of federally funded hemophilia treatment centers, and it would be good for them to know. I know you have a plan to educate them.

The most difficult part about treating hemophilia is the additional providers who fall outside the hemophilia treatment center team. Those would be internists and dentists and others who treat what turn out to be complications of hemophilia.

Almost every patient has bleeding into their dental area, if it is not well controlled. Those are the physicians, especially those physicians who are treating our medicare and medicaid patients, that I am most concerned about getting some kind of formal communication from the FDA.

Those are the physicians that are going to be most likely. They are already underfunded for treating these patients. Those are going to be the physicians that

are most likely to walk away from treating them.

So, I don't know if it is so much of a question as it is a plea that some kind of -- that we develop some kind of communication specifically for that physician, and that it be distributed through medicare or medicaid communication lines, so that there is some kind of pre-communication to them.

There are going to be two kinds of confusion that come out of any kind of announcement. One, they are going to see hemophilia and not recognize XI, which is a very small percentage of the population.

The second is they are going to see hemophilia and say, you know, I don't get paid for this patient anyway. So, why should I continue to treat them, especially if I am putting my practice at risk on some level. So, that would be my advice, comment input.

DR. WEINSTEIN: I appreciate that. I do point out, of course that we are, first of all, concentrating on the interaction between the IND holder and the specific factor XI patient, targeting this very small, hopefully well defined population here, but your comments are well made here.

There will be comments about the variant CJD and hemophilia treatment, factor VIII directly related to the potential variant CJD in factor VIII products. Again, this

would be an issue that we would bring forth to the committee at a later time.

MR. BIAS: And it all could go very smoothly, unless it is a slow news day, and then we could see it 15 times over every news network, and it could be a hemophilia crisis because they are not going to get it right. They are not going to indicate that it is a small group of patients.

They are going to say, hemophiliacs, hemophilia on the ticker tape. It is going to be the story of the day, depending on what is going on. So, just keep that in mind as the communication plan moves forward.

DR. HAMILTON: I would just endorse that, but add a PS to it and say that, while medicare and medicaid are probably going to be the first physicians to react in that way, because all models of reimbursement follow medicare and medicaid, it is going to go to the private pay sector as well.

It ranges from pediatrics to orthopedics to everything, every different area of medicine that treats a patient like that.

I realize that you are going to start out with the small cadre of patients with the IND and the IND holders and so forth, but as Val said, if it is a slow news day and word leaks out, then you are going to have all these other doctors talking about it, the patients are

going to be talking about it.

Somebody is going to say to a patient, well, I heard, you know, and then here we go and we have a mass communications hysteria on our hands.

DR. COLVIN: Just a question about how sort of factor XI was used. How many donors' plasma was used to manufacture the factor XI, just out of curiosity. Was it similar on scale to how many donors are used to make factor VIII?

DR. WEINSTEIN: We estimate -- we described the model earlier. I think it was about 20,000 donors per pool.

DR. COLVIN: Is the pool a realistic one, or is that the industry based estimate that is a factor of 10 off?

DR. WEINSTEIN: It is pretty accurate.

DR. COLVIN: Then was factor XI used in an ongoing way, sort of like factor VIII is used?

DR. WEINSTEIN: It is not used in the regular chronic fashion. It is used mostly in the preventive field for occasional bleeding. Factor XI deficiency is much different.

DR. COLVIN: It is a much more mild disease. Has the IND been approved for this?

DR. WEINSTEIN: There are several INDs that were used between 1989 and 2000. It is not a problem in that

sense now.

DR. TELLING: Thank you, Dr. Weinstein. So, next we are going to hear from Dr. David Asher. He is going to summarize discussions of the World Health organization relating to distribution of infectivity in tissues of animals and humans with TSE.

**Agenda Item: Summary of WHO Consultation on Distribution of Infectivity in Tissues.**

DR. ASHER: I can't really summarize in 20 minutes a proceeding that took two and a half days, produced a 53-page document, small print, that just issued, and involved presentations by 42 speakers, but I will do the best that I can under the circumstances.

The consultation is one of a series that was organized by Anna Padilla of the WHO secretariat over the past number of years. She is aided by others in her section, but she really is the prime mover.

The single most important thing I can hope to show you is that the document is readily available, either directly through this PDF that is on the little handout, or through a link in the WHO web page.

In the handout, what I have done is to put in the slides condensed a little bit, that include things that were actually covered in the consultation.

I will show some other material that is

explanatory, because it is very hard to follow the reason for the consultation, I think, without either reading the whole document or with a little explanation.

The purposes of the consultation were several. Specifically, the goal was to revise a 2003 consultation document on the safety of medicinal products, particularly biological products, with emphasis on vaccines and products derived from human blood, with a little attention to human cell tissues and products derived from them.

To support that, scientific information, particularly during the past two years, was reviewed, risk assessments provided to regulatory authorities in several countries.

One important goal of the consultation was to provide advice to national regulatory authorities with limited resources.

The WHO in general does not expect that its consultations will be particularly -- I don't mean to say valuable, but they don't have the same weight in countries that have elaborate approaches to the TSEs, like the United States, Canada, the European Union, Japan, Australia and New Zealand.

They also don't expect to get underdeveloped countries are going to be able to undertake some of the steps that we have taken in our countries, but they do

believe that, for reasons that I will show you in a second, that the countries that don't have the resources that we do could nonetheless be aware of the issues because of the nature of the risk.

A special goal that we will end with, and you will hear a little more about, I am sure, during the open public hearing, was to summarize the available information about the distribution of infectivity and abnormal prion protein in tissues and body fluids in the TSEs that the WHO deemed most important, which are Creutzfeldt Jakob disease in humans and the natural diseases of ruminants.

There has been no attempt made to summarize the huge body of information on rodent models, more because it has been a North American problem, was chronic wasting disease, or mink encephalopathy, for that matter addressed.

There was an ambitious agenda summarized in the document. I won't go through everything, although I made three or four slides that summarized points that seemed to me to be of particular interest. I will go through briefly the TSE infectivity table at the end.

The reason for the concern is the continuing recognition of BSE and variant CJD in countries throughout the world.

There are now 26 countries that have recognized BSE in native cattle and, although the epidemics do seem to

be declining, two countries, two new countries, recognized cases this year.

The United Kingdom, which has had more than 184,000 cases, still had 225 cases last year, and cases have occurred, as you know, in North America. We hope that the cases in Canada are going to turn out to be largely restricted to western Canada. As far as I know, there have been no cases in Ontario, Quebec or the Maritimes, but it bears watching.

Another risk is that caused by the export of contaminated meat and bone meal from the United Kingdom during the years of concern throughout the world.

This map is based on UK customs and excise records that show that contaminated meat and bone meal is at least on record as having been exported to a number of countries. It wasn't exported, some small amount, by the way, to the United States and Canada, but also to countries that haven't recognized BSE, like the former Soviet Union, Indonesia, Thailand, and one doesn't know what those countries did with the meat and bone meal. There is some possibility that they introduced BSE and have not recognized it

Another risk comes from travelers. We know that people have been infected with variant CJD in the United Kingdom or perhaps other countries, and then returned to

their homes bringing the infection with them, and the transfusion risk presumably travels with them to their home countries.

So, it was felt that this disease has become a global issue, and all countries should at least be aware that the disease exists.

Now, how does it compare with AIDS and tuberculosis and malaria and avian flu and automobile accidents and all the major killers?

Obviously, many of these countries are not going to be able to do much in the great scheme of things, are not going to be able to invest in defending themselves against a relatively remote risk, but at least they should be aware of it.

Also of concern, it wasn't this goat, but there has been recognition in the past couple of years that BSE, at least as defined by recent profiles of putting extracts into mice, BSE has not only been transmitted experimentally to small ruminants like the sheep and goats, but there is now evidence that one goat in France appears to have been infected and possibly one in Scotland.

that means that contaminated feed presumably got used in Europe to feed these animals, and in many parts of the world small ruminants are a much more common food source than are cattle.

The natural history of this disease in small ruminants seems to be quite different than that in cattle. The two phrases that got amputated from the bottom of one of the slides in the handout that referred to the transmission of BSE to sheep and goats.

Also, there remains a concern about variant CJD. Some of that was explained by Dot Scott earlier today. Although the clinical disease has peaked in the United Kingdom about eight years after the peak of BSE in cattle in the United Kingdom, the finding of the two positive appendices suggests at least the possibility of the prevalence of undiagnosed cases there may be higher than was expected, and one doesn't know how long before the appendix becomes positive the blood becomes positive.

So, there is an unknown blood risk in the BSE countries that is of concern, and the three transfusion transmitted infections, the first one recognized in December 2003, only increased that concern.

The growing number, relatively rapidly growing number of cases in France, 17 of whom had no substantial history of time spent in the United Kingdom, is another source of concern, as is the Japanese cases, that Dot mentioned, who spent less than a month in the United Kingdom.

There is never any reason from animal studies to

doubt that a single exposure might, under adverse conditions, be enough to infect a person, but it reminds us that there is a residual risk that we haven't dealt with by deferral factors.

So, those are the reasons why the World Health Organization remains concerned, hosts these consultations. It is gratifying that, except for blood, no other new class of medical products has been recognized as transmitting CJD in the past 10 years, but here have been now over 360 recognized iatrogenic transmissions.

One of the things that was attempted at the consultation was to simplify, for those of us who are more public health oriented, the nomenclature regarding abnormal forms of the prion protein.

Jiri Safar discussed this this morning, the various forms of abnormal prion protein protease resistant, protease sensitive, degradation products of different lengths.

We didn't get into the five or maybe six subclasses of prion proteins in the Cohen classification scheme or the four, maybe it is five now, in the Parker Gambetti scheme. It became so difficult just to talk to each other that it was proposed that, for our purposes, the term PRP<sup>TSE</sup> be used for all the abnormal forms of prion proteins, regardless of their molecular nature, and let the

specialists, in the end of days, figure out what those forms were.

So, for our purposes, we used that instead of PRP<sup>SC</sup> or PRPRES, although UK authorities, before the end of the meeting, had started referring to PRPD for disease related PRP. So, go figure. At least for the rest of this talk, we will use PRP<sup>TSE</sup> .

I went through the proceedings and I selected some points that I thought were of particular interest to this group today, and I list them on the next three or four slides:

Naturally affected cattle, infectivity detected by mouse assay demonstrated only in brain, spinal cord, retina and a pool of nictitating membranes, but not in pools of lymph nodes.

Infectivity was detected in some peripheral nerves, and a solitary muscle of a single case of BSE in a German cow. That is a greater concern in Europe where there is more BSE than the maximum of one case per million, we hope, that USDA estimates here. There was only infectivity found in a semi-tendonsis muscle using only an extremely sensitive transgenic mouse that Martin Groshup(?) has developed.

Still, it would be disturbing if meat itself was intrinsically infected rather than neural tissue, because

contamination has been the thing that is of greatest concern for the safety of the food supply.

Cattle experimentally exposed to oral route BSE infectivity detected in distal ilium throughout the course of disease after six months in the palatine tonsil, and only in cattle assay, demonstrating that the mouse assay, except for some transgenic mice, is considerably less sensitive, and this is the part that got amputated from your handout, BSE experimentally transmitted via the oral route to sheep and goats and one, possibly two, goats now recognized in Europe to have been infected under field conditions.

No sheep has been recognized, but they both got fed similar, presumably some similar feeds having presumably some level of contamination.

Under specific experimental conditions, brains of some TSE infected rodents may be infectious by bioassay, while TSE remains undetected. That problem has been mentioned here today.

Immunoassays detected PRP<sup>TSE</sup> in brain of BSE in cattle at least three months before onset of illness. No immunological method yet validated as sufficiently sensitive to detect PRP<sup>TSE</sup> in the blood of infected animals or humans, although promising initial results were reported by several groups of investigators and we will be hearing

tomorrow reports by all six of those groups who reported last year, plus a report by Dr. safar.

Transfusion experiments have not been conducted in cattle, although studies using small amounts of blood and spleen of cattle assayed in mice and cattle, in operating cerebrally, failed to detect infectivity.

The regulatory conclusion to that, a conservative regulatory approach would assume that bovine serum might potentially contain TSE infectivity, presumably, in small amounts.

Ruminant blood, blood derivatives such as fetal calf serum in cell culture, and bovine serum albumen have not been identified as a source of infection, but should be properly collected to reduce the risk.

However, blood of sheep with both experimental BSE and natural scrapie can be infectious and, because scrapie and BSE agents behave similarly in sheep and goats, the blood of small ruminants should either be avoided in preparing biologicals, or selected very carefully.

I think because sheep and goat blood are used as a source of immune sera, some quite useful sera, the second criteria is more feasible, at least on a worldwide basis.

There is a continuing need to ensure that all regulatory authorities with limited resources have ready access to reliable and up to date information when

assessing TSE risks and evaluating medicine product safety. Those were one of the main reasons for the consultation.

Although I am not going to review the 42 talks, I think that I found comparisons of the results of the natural risk assessments for variant CJD in blood to be informative, because they seemed to be, although independent in their design, they seemed to come up with very similar results.

For example, Marc Turner presented an assessment for the United Kingdom suggesting that one in 120,000 transfusions there might be from donors incubating variant CJD.

If strict indications for transfusions were observed, the benefits clearly exceed the risk. One of the take home messages is, as in many other areas of medicine, if you don't need to use a product, don't, and then there won't be any risk assessment associated with it.

Plasma derived products, their estimates suggest that minimal risks, surgical and dental instruments, human cell tissue products, the risk is highly uncertain. That was reviewed by Peter Bennet.

Touvin presented an independent risk assessment from France that yielded virtually identical results as those reported by Marc Turner. So, it is reassuring to see two different risk assessment teams come up with very

similar results.

For Australia, the Farrugia report, they identified factor VIII as having the most risk among the human plasma derived clotting factors.

Susie El Saadany stressed how difficult it was to communicate these risks, as we have discussed heretofore, due to all the uncertainties.

Johanne Lower from the Paul Erlich(?) Institute, presented an extremely complex risk assessment, but there was also -- I can't pretend to have understood the methodology, but I thought the results were interesting.

They concluded that, under realistic conditions, for Germany the disease should not become an endemic infection, presuming that they get rid of the BSE. It should not become an endemic human infection maintained by blood transfusions.

Transfused donors reduces the risk in Germany only slightly, but the German conclusion should not be applied to other countries that have different BSE and variant CJD risks.

Finally, as Dot Scott reviewed for you today, due to uncertainties in the assumptions -- you will hear more about that in the next meeting also -- it is very difficult to offer confident predictions regarding the probability of variant CJD infections, not to mention illnesses in people

exposed to various blood components and plasma derivatives.

Let me close by going over the tables of infectivity in human and animal tissues and other material. This was revised from the previous table by a very energetic working group chaired by Paul Brown, who is here today, with Gerald Hills and Ray Bradley handling the animal tissue distribution.

I took the liberty of asking one of the more artistic members of our group, Olga Maxiomva, to translate the table -- it is not as colorful in this publication -- into a color tile table.

It is very difficult to see the whole gestalt of those tissues that are either infectious or contain abnormal PRP and those that don't and those that haven't been adequately tested, from a black and white table.

So, we are presenting - and I divided them up a little bit, just to make them easier for you to see. So, those issues in which infectivity or PRP<sup>TSE</sup> are detected I will present in red.

Those in which there is no detectable infectivity or PRP<sup>TSE</sup> in some reasonable number of experiments as judged by the working group I present in dark blue.

Those that are either not tested, not applicable or for which data are limited or are preliminary are in light blue.

When we come to the issue of blood, with the exception of sheep with scrapie and BSE and humans with variant CJD, the results were really quite controversial, contested, at the time of the meeting.

It is hard not to be enthusiastic when you see some of the results that were presented, and that is not in your handout. You are going to see better data tomorrow, but I put it in this presentation.

The preliminary data looked quite good. On the other hand, the BSE community has been bitterly disappointed by reports of detection by PLP TSE tests of agent in blood before, and there was a certain reluctance to accept without further confirmation some of the preliminary results on blood tests. Again, you will be seeing that in some detail tomorrow.

So, high infectivity tissues are those either in or anatomically associated with the nervous system. As you can see, most of the tests with tissues clearly contain infectivity. As you know, a lot of that is in relatively high titer.

Lower infectivity tissues, there are also quite a few positives, although some of them are relatively consistently negative.

One of the things that strikes me, just from looking at the table, is that if you only saw part of this

table, how difficult it would be to predict from one animal model -- for instance, these results from low infectivity tissues from sheep and goats, what you are going to see in cattle.

It is interesting that the situation in sheep and goats seem to be more predictive of the variant CJD matter than was the picture in cattle.

Going down the list, other lower infectivity tissues, and here are the blood tests for PRP<sup>TSE</sup> tests for variant CJD or other forms of CJD, BSE and scrapie in sheep and goats that we will be hearing about tomorrow.

I just want to show you what I mean by the really exciting preliminary results that elicited two kinds of results, one high enthusiasm, and the other concern that they looked almost too good to be true.

This was the first test presented, and you see that at least the claimed sensitivity overall -- that is, the number of infected sheep, hamsters, cows, mice, monkeys and humans claimed was 100 percent of those tested, if we at least got the numbers down correctly.

Where there are missing data, it is not because the developers that tested didn't know. It is because we somehow didn't get it and the data wasn't provided when Jean Phillip was preparing the summary in the same dose for the spike limit of detection.

The specificity also looks amazingly high from the preliminary results. Only one false positive out of more than 100 bloods tested, five other tests, a total of six. Taken together they are not quite as astounding as the first one. Still, overall, they look very good, particularly the presented specificity.

Again, it is hard not to be enthusiastic, but we have been burned before. So, the consultation felt that we should really wait for confirmation of these results before accepting them as bona fide detections of PRP<sup>TSE</sup> in blood.

A number of issues, all attempts to detect either infectivity or abnormal prion protein have been negative, although I warn you, if you look on here you see an awful lot of light blue, not tested, which shows you how much additional work would be needed before one could be at all confident that there is an anti-infectivity in those tissues, absolutely.

These are my own caveats. Limitations of these studies come from the small numbers of human cases and animals studied, the small numbers of bioassays attempted, the small volumes of material sampled, the limits of detection usually unknown, some of the bioassay animal species, as you saw, with outbred mice, with conventional mice with BSE. Some of them are relatively prion sensitive.

The PRP<sup>TSE</sup> assays vary in sensitivity, some of

them relatively insensitive, very limited numbers of infected animals studied during the incubation period.

Infected human beings almost never identified and studied before the onset of overt disease. Not unimportant, the uncertain relevance of one animal TSE model for predicting another animal TSE distribution of infectivity, or the human distribution of infectivity.

I will close with the credits. The chairman of the consultation was Pim van Aken from The Netherlands. Four of us assembled the report with a great deal of help from those listed in the second block down.

Paul Brown, Ray Bradley and Gerald Wells worked very hard to revise these tables. Nothing is ever finished, and I have an idea a couple of years from now we are going to see some revisions to these tables. If there are any questions, I am happy to answer them. If not, thank you.

DR. TELLING: Thanks, Dr. Asher. There is a question here.

DR. MANUELIDIS: What type of TSE tests were used in these ones that look so spectacular?

DR. ASHER: You will hear all of them, from the actual -- that is why they are not in your handout. I think that the folks that developed them ought to be able to present their own tests and their own data tomorrow.

We have a whole session on tests and we invited

everybody, every group, who had been represented at the WHO consultation, because we don't like to play favorites.

We invited everyone to come, everyone agreed to come, and we look forward to seeing what has gone on in the past year, whether these things are continuing to develop in a promising way. Are we getting close to a blood based screening test or is there more -- well, of course there is more work to be done, but how close are we. You will have a chance to judge for yourself tomorrow.

DR. TELLING: Thank you, Dr. Asher, very much for that elegant summary. The final item on the agenda is the second open public hearing. Dr. Freas, will you let us know who is registered for this open public hearing session?

**Agenda Item: Open Public Hearing.**

DR. FREAS: Again, this is the time for members of the public to address the committee on issues pending before the committee.

I have received two requests and they will be called to the podium in the order in which I received the requests.

The first is Dr. Charles Sims, medical director, California Cryobank, Incorporated. While Dr. Sims is approaching the podium, our chair has to read the required and mandatory statement for the open public hearing.

DR. TELLING: Both the Food and Drug

Administration and the public believe in a transparent process for information gathering and decision making.

To assure such transparency at the open public hearing session of the advisory committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement, to advise the committee of any financial relationship that you may have with any company or any group that is likely to be impacted by the topic of this meeting.

For example, the financial information may include the company's or a group's payment of your travel, lodging or other expenses in connection with your attendance at the meeting.

Likewise, FDA encourages you, at the beginning of your statement, to advise the committee if you do not have such financial relationships.

If you choose not to address this issue or the financial relationships at the beginning of your statement, it will not preclude you from speaking. So, Dr. Sims?

**Agenda Item: Statement by Charles Sims.**

DR. SIMS: I am Charles Sims, president of the California Cryobank. By training and background, I am a

pathologist, not what many of you would consider a scientist, since most PhDs don't consider MDs seriously in terms of science.

I have no expertise in prion disease. I do have expertise in the field of reproductive medicine, in particular the area of reproductive tissue banking.

I am the founder and the medical director of one of the largest human sperm banks in the United States. I have been active in this field since 1977.

The company paid for my trip here. Since I am one of the principals of the company, it comes out of my pocket. I have two conflicts of interest. One is that, as we are speaking at this moment, I have an FDA inspection going on in my laboratory. I am not sure which is the least pleasant of the two, but I appreciate being here.

My wife thinks that I spend too much time traveling for business and not enough time traveling for pleasure. So, those are my two conflicts of interest.

I am here to discuss and request that this committee and the FDA reconsider the guidelines that are currently being used for human sperm and egg donors.

The current guidelines were developed, I believe, from everything I can read, to address the issues of blood and blood products, not the issues of sperm or egg donors.

It is our opinion, in the field of reproductive

medicine, that those standards and those criteria and those guidelines are not appropriate in this area.

Sperm donors is not a commodity like blood. It is not a commodity like plasma products. It is not a commodity like demineralized bone. Doctors don't choose a sperm donor as a product.

The women themselves who are the recipients of these are the ones who select the donor. They select an individual donor. They do not select a sperm donor. It is a very important difference in those two.

To the best of my knowledge there has been no transmission of CJD or any other type of transmissible spongiform encephalopathy by sexual relations or activities.

When the guidelines by the FDA were originally issued and I became aware of them, the applicability to reproductive tissues -- namely egg donors and sperm donors -- at the time I thought that the influence on that would be relatively trivial. I was wrong.

In 2006, when we applied the guidelines to reproductive tissue donors, sperm donors in particular, we lost 20 donors out of our program. That amounted to about 13 percent of the active donors in our program. That is a material effect.

It may seem trivial to the rest of you but, to

us, it is not trivial. It is very, very difficult to recruit sperm donors.

There has been a lot of negative publicity and media about donors losing their anonymity, and it is becoming increasingly difficult to attract and retain donors.

A blood donor can simply walk into the blood donor center, take a short history and physical, check the hemoglobin, and then they can accept it as a donor, and that is the end of the story.

A sperm donor, it takes weeks and sometimes a few months to fully qualify as a sperm donor. It is a long elaborate process. We know more about our sperm donors than I would say most of you knew about your spouses when you got married.

We do a three generation medical and genetic history, we do extensive testing over a period of time. We wind up, out of 100 donors who apply to become donors, only two or three actually wind up being in our final donor pool.

So, we lose something like 97 or 98 percent of the potential applicants, the potential applicants. We lose them for a large number of reasons. The single largest reason is probably poor sperm production.

The fertility parameters are suboptimal for our

purposes. They are normal for normal reproduction, but they are suboptimal for sperm banks. Because of the freezing and thawing, we lose about 50 percent of the motile cells when they go through that process. So, we need to start out with higher reproductive parameters than average. We lose them for many reasons.

Each one of these small barriers, which may seem trivial, they all add up. The cumulative effect of that is that it is getting harder and harder and harder.

I am the immediate past chair of the reproductive council of the American Association of Tissue Banks. I just conducted a national survey of all the sperm banks in the United States that provide donor semen. So, I am very familiar with the issues that the directors of those programs have.

Universally, they tell me that the single largest problem they have is finding and keeping sperm donors. It is not an easy task.

To have additional barriers placed on us, such as the exclusion of donors who lived in the United Kingdom for six months or three months or a military base in Greece for six months or some place else in the United Kingdom for five years seems trivial, I am sure, to many of you but to me it is not trivial.

It has been painful, it is hard. It is hard to

replace these donors. I think we may have had more than the usual number of donors of that, because of the places that our laboratories are located. They are in cities with a more cosmopolitan and perhaps more widely traveled group.

I would like to explain and give you just a little context about sperm banks and sperm donors and what this goes through.

First of all, I would like to address the issue of processing sperm. We don't pool sperm. You pool blood, you pool a lot of things, but sperm is never pooled. Egg donors are never pooled. So, all of these are very individual things.

Nearly all the inseminations done in the United States are done with the intrauterine method. The intrauterine method achieves about twice the fertility rate per cycle that intracervical inseminations do. That is the reason why nearly all specialists in this area have moved from cervical insemination to intrauterine insemination.

In order to do the intrauterine insemination, you have to remove the seminal plasma. The reason you have to remove the seminal plasma is because seminal plasma contain prostaglandins, causes uterine contractions.

So, they can be trivial or they can be more severe. So, that is universally done. Most and much of the sperm washing that is done to remove the seminal plasma

also removes leukocytes.

If you use density gradient methods you remove nearly all of the leukocytes as well as the seminal plasma. You also get rid of the non-motile fraction of sperm to a large extent using the density gradient methods.

If you look at the biomass or the bioburden that donor semen provides, it is about -- I would estimate that it is somewhere between an eighth to a tenth of what a normal human ejaculate has.

The normal human ejaculate has three to four ml of volume and we take that, we remove the seminal plasma, and we re-suspend the density gradient cells, in our case, into a sperm media that has cryoprotectant and other buffers in it.

That winds up being only about a tenth or so of the original volume of ejaculate. In normal sexual relationships, the exposure or bioburden is nearly 10 times that.

There is far more normal sexual activity that goes on in the country and the world than there are sperm donations. There have been no reports, to my knowledge, of the transmission of any form of CJD by sexual transmission between spouses or others, and there has been none as far as donors are concerned.

It is for this reason that we feel that the use

of the blood donor criteria is inappropriate for sperm banks. My friend, David Mortimer, published a paper in Reproductive Medicine on line just this past week, which you have in your handout, which lays out basically the basic arguments for asking for this exemption from the blood products and blood guidelines.

I would be happy to answer any questions you may have about sperm banking. I am not capable of answering detailed questions about prions.

DR. TELLING: Thank you, Dr. Sims. I will entertain questions with regard to points of clarification, if there are any right now.

If there are none, thank you once again, and I would ask Dr. Brown, representing Nordisk Cryobank, to come and speak to us.

**Agenda Item: Statement by Paul Brown.**

DR. BROWN: Thank you very much, Glenn. It is a little disorienting to speaking to the group from here. I used to speak from there and be the judge.

A month or so ago I got an e mail from a Danish company. As you know now, it is Nordisk Cryobank, and they wanted me to talk to you today.

I sent them a message back saying, well what do you want me to talk about. They said, well, we would like you to talk about restrictions on importing our product.

What is that? Well, the product is sperm. I said, well, I don't really have anything much to do with that. Besides, I said, the meeting that you are going to is basically all about plasma. It is really not concentrating on tissues or cells.

They said, well, what about the WHO guidelines? ? That seems to be relevant. I thought about it for a little while.

I decided, like an attorney accepting a case to prove a principle in front of the Supreme Court, that I would take on the request. So, I have, in fact, come to you at the best and in the employ as a consult of the Danish Cryobank that historically has contributed sperm donations to the United States.

In the context of full disclosure, I should probably mention to you three unhappy facts. The first is, I have never made a donation myself.

The second, worse, I have never been invited to make a donation. The worst of all is, my banking days are long past. So, with that preamble, and to make this as painless as possible, what I am going to do really is just read into the record the handout that you have in front of you.

In June 2002, which you have heard, the FDA published a draft guidance on human cells, tissues and

cellular and tissue based products, in relation to the risk of contracting variant Creutzfeldt Jakob disease. This guidance has still not been finalized.

In September of last year, it should be actually, not 2006 but 2005, the WHO held their consultation that Dave has just summarized for you. It included the table that Dave also has referred to.

In point of fact, the risk from variant Creutzfeldt Jakob disease tissue is a function of several things.

It is a function, like any other infectious disease, transmissible disease, it is a function of the geographic location of the donors, to what they may have been exposed, the level of infectivity in the tissue, obviously, any reduction of that infectivity that will be the result of processing.

It also has to do with the route of administration. We all know that if you give something that has CJD agents in it directly into the brain you will do a good deal more damage than if you are giving it from any number of peripheral routes, and finally, it is a function of dose.

You know from work on blood that you can transfuse an infection into a sheep using 200 ml of plasma or whole blood intravenously far more effectively than you

can by inoculating 0.03 ml of buffy coat into the brain directly.

So, all of these factors have to be taken into consideration in a risk assessment. The example that I have been asked to present to you is the excluded tissue of sperm.

The guidance that is still in draft form from 2002 recommends the exclusion -- actually really only addresses one of those factors and it is the geographic source.

They recommend, as you well know, the exclusion of donors with a total residence time since 1980 of three months or more in the United Kingdom and five or more years in all countries in continental Europe.

One consequence of this is the exclusion of all cells, tissues and tissue products from the entire European native population, obviously.

So, looking at the five factors to see how realistic this exclusion is, Denmark has had a thorough systematic active surveillance of BSE for seven years and CJD for 10.

During this time, there have been a total of 14 cases of BSE, decreasing numbers each year, and this year none at all.

Around 54 cases of CJD that have been identified,

none have had the variant form of the disease. As a matter of fact, outside of France, there have been only five cases of variant CJD identified in the entirety of continental Europe with a population on the order of something like 300 million people.

So, it is really no longer sensible to group France and the rest of Europe in a single basket. It is also worth noting that, during the same period, Canada has had nine cases of BSE including one in 2006, no cases of variant, as in Denmark, but the FDA has not thought it necessary to restrict donations from the Canadian population.

The second point, tissue infectivity levels. The WHO panel did construct a table and showed that all experimental attempts to transmit disease from reproductive tissues, including semen, from cattle with BSE and from sheep with scrapie have failed.

These have been substantial experiments. Limited experiments in humans have also failed. More expensive examination of the pathologic prion protein have been done and have been uniformly negative, both in animals and in humans.

I think even stronger support for the notion that semen and sperm in particular is not transmissible comes from the epidemiologic observation that there has not been,

despite decades of searching, a single proven instance of vertical transmission.

The USDA, interestingly enough, permits the importation of bovine sperm even from the United Kingdom, if several criteria are met that minimize the possibility of an infected donor. Those criteria do not include a bovine travel history.

The third point, tissue processing, you have just heard and I just summarize or recapitulate the processing of semen into sperm, both in Denmark and in the bank that you have just heard about, involves a density gradient which reduces leukocytes from one to two million maximum per milliliter, to undetectable levels of 10,000 cells or less per ml. That is below the threshold that is acceptable for leukodepleted blood.

In addition, after it is washed, and as you probably have heard from Bob Rohrer, there is some evidence that simple washing will reduce the amount of infectious agent attached to cells in the blood.

The fourth point, route of administration. Venereal transmission of disease has not been adequately studied experimentally, but epidemiologic evidence indicates that it does not occur.

Finally, the total amount of administered tissue, as you have just heard, is about a tenth of a single

ejaculation, about a half an ml in volume.

It is important to know that you can actually have tissue that is infectious and give it, as I mentioned earlier, in a dose that is too small actually to be transmitted.

That can be overcome. In a very nice paper a year or so ago, that can be overcome by administering repeated subtransmissible doses, but insemination is a one shot procedure.

So, a consideration, it seems to me, of all of this information leads to the conclusion that sperm donated from healthy, young individuals living in Denmark does not pose a risk of variant CJD transmission.

Equally important and more general, the FDA donor exclusion criteria really do need to be revised to reflect both the change in BSE vCJD situation in Europe and the most recent information on tissue infectivity. Thank you very much.

DR. TELLING: Thanks, Dr. Brown. I will once again entertain questions related to clarification.

DR. MANUELIDIS: Actually, I sort of agree with that. I think that it is important to take cells, cells of the body, et cetera, as a very separate type of issue from blood.

There are many types of cells. Just to be equal

about it -- as Paul knows, I like to be equal -- we haven't considered egg cells either.

Actually, there is one thing that confuses me. We did lots of experiments with guinea pigs, with insemination, and we kept them for 12 years, and not a single case of insemination or cohabitation with CJD infected guinea pigs with each other led to any kind of infection.

However, in 1989 -- maybe you have a follow up for this -- when I was in the United Kingdom and they were talking about the spread of BSE, it turns out that they had sent -- they felt that the source of the BSE in Saudi Arabia, they had sent a breeding bull to Saudi Arabia, and that is what they felt was the source of the Saudi Arabian BSE cases.

So, maybe you could follow up on that. I think if that were ruled out, that would also be a very interesting point.

DR. TELliNg: I am just going to make this point. Because this is an issue that is not on the agenda, we are not really at liberty to discuss it in any depth today.

I did want to give you the opportunity for questions of clarification. If there is a question of clarification, then I will entertain it, but otherwise maybe we should move on.

DR. MANUELIDIS: I just wanted a clarification about the case that was sent to Saudi Arabia.

DR. SIMS: I have no clarification, and I think any American effort to get clarification from Saudi Arabia, at this point in time, will fail. Perhaps in the future it will succeed.

The reason that this presentation has been made, at least from my point of view, is that I do think it is a good case study in why you are going to have to re-address, we are not going to come back to a consideration of excluding European tissues and cells on the basis of information.

The anchor to this meeting was today's discussion. If you have no evidence of infectivity in the tissue, it just strikes me as unreasonable to group it in with tissues for which you do have evidence.

DR. TELLING: And the FDA is happy to listen to these comments.

DR. SALMAN: This is to clarify about the Saudi Arabia semen, which was rejected, that hypothesis. The main source for that infection was the importation of the MBM from the United Kingdom.

DR. TELLING: Thank you, Dr. Brown, and thank you for your full and frank disclosure also. So, with that, I would like to thank everybody once again.

DR. FREAS: I do have two quick announcements.

[Housekeeping/logistics matters discussed.]

DR. TELLING: So, the meeting is adjourned. Thank you very much.

[Whereupon, at 3:55 p.m., the meeting was recessed, to reconvene the following day, Tuesday, September 19, 2006.]