cows or in mice that there's a 10^{-2}-10^{3} there. However, I would like to know, first, in the RIII mice, are they special? Can they be improved by genetic manipulation?

Also, what is a Delphia test, and what is the prionics test? I think all those would be helpful to clarify.

DR. WELLS: On the question of the mice, RIII mice and C-57 mice, they are the short incubation period, the homozygous sync gene or the short incubation PRP gene mice that are used -- the conventional mice that are used for all the assays and part of the panel of conventional mice strains that are used for assays of both scrapie and BSE.

You're quite right that there are studies now that have carried out transgenic studies of infectivity in transgenics. But I'm not aware of any of these that have actually standardized the procedure to a degree that would enable us to use them in a routine manner.

CHAIRMAN BROWN: Yes?

DR. GRIFFIN: Well, have they been directly compared? Are they more sensitive than RIII or Black 6 mice? I mean, is that direct comparison --

CHAIRMAN BROWN: Maybe Gerry knows more

than I do, but to the best of my knowledge, the RIII mouse remains the most sensitive assay animal with the shortest incubation period.

DR. GRIFFIN; So transgenesis into that mouse does not improve things.

DR. WELLS: That's our primary transmission.

DR. GRIFFIN: I think the real question is -- I mean, I have the same question as Alice's. What's the sensitivity of the assays, all these various assays, and can they be improved; because it sounds like that's really a limiting factor in trying to --

CHAIRMAN BROWN: That is, the question goes to whether or not the demonstrated tissues or the tissues that have been demonstrated to be infectious could be, shall we say, the tip of the iceberg.

DR. GRIFFIN: Exactly.

CHAIRMAN BROWN: There might be low levels of infectivity that haven't been picked up yet. Right. Well, I think the answer to the question is maybe not the tip of the iceberg, but certainly low levels of infectivity might be present, if you are assaying in any species that is not the host species.

On the other hand, you have seen evidence

presented in which the species barrier has been eliminated by using cattle as the assay animal, and the results of those rather extensive assays are very optimistic. That is, you can't get better than cattle and cattle.

DR. HUANG: How about the prionics test and the Delphia test?

CHAIRMAN BROWN: Go ahead, John.

DR. WILESMITH: I'm not an expert on these tests. These are two of four tests which were put through an EU evaluation. I use the word carefully, not a validation. Briefly, the prionics is in kit form, and it's basically in an ELISA format. Okay?

The prionics uses this Delphia technology which I really am light on, but developed by a company called Wallach, and it's this rapid -- sort of detecting this rapid fluorescence that one gets from the test system.

So they are basically trying to detect various forms of PrP-SC, and that's the interesting bit, I think, that we're learning from these tests, because they are all actually detecting different forms, aggregated and progenase K resistant. I think we might actually get some information from comparing these tests on, if you like, the pathogenesis of the

development of abnormal forms of PrP.

So they are in use and so on, and the prionics is the most attractive if you want to process a lot of samples, but it's no better than a good pathologist.

CHAIRMAN BROWN: If you want to make the Swiss rich, prionics is the test. As a matter of fact, they are all good tests, and several of them depend on an ELISA format with refinements of detectability. Some would argue that the Western blot remains the gold standard. Immunohistochemistry, in some cases, in different studies you get a little -- you get an occasional positive immunohistochemically that doesn't turn up in a Western; vice versa.

There is an immunoblot. There is another technique now which takes formalin-fixed tissue and converts it into a kind of immunoblot. There are a half-dozen different technologies out there, and they have varying degrees of sensitivity, but in general the sensitivity is certainly equal to the same tests used on conventional infectious agents.

DR. ALMOND: Mr. Chairman, could I just add that I was on the EU committee that was established to validate those four tests. The validation was carried out by the importation of 1,000

clean brains from one of the clean countries, and that they were randomly mixed blind with 250 brains that were provided from confirmed cases from MAF in London.

Three of the four tests identified 100 percent of the positives and all of the negatives as such in the way the material was presented. So they were very good tests.

The only caveat we should add, which John has already alluded to, is that these animals from which the positive brains were taken were all clinically sick animals and, of course, therefore, terminal. They had been confirmed by histopathology.

None of the tests were assessed in terms of their ability to detect preclinical animals. However, each of the tests were tested with dilutions of brain macerate where the dilution was of an infected brain by uninfected brain. Some of the tests did rather better than the others in terms of the dilution to which you would still get a positive with those brain macerates.

It was absolutely clear that what was required to follow up on those tests was a diagnosis of the animals that were in the pathogenesis study that Gerald Wells described, so you could then assess how the tests were performing on preclinical animals.

In that sense, the sort of comparison that John talked about will be useful at the moment. But as far as the tests today are concerned, they have only really been tested on clinically sick animals.

CHAIRMAN BROWN: Yes. The other part -- We're going to have to stop again, but the other part of the question, of course, is we're talking now about optimum tissue, brain tissue. When you're talking about muscle, when you're talking about plasma, you may be in a somewhat different situation, and none of these tests are as sensitive as a bioassay in detecting very low levels of infectivity. I mean, that's a fact.

All right. We'll move on now to the final -- not the final, actually -- to a presentation about the European Union approach and perspective. I have two speakers listed. The first is Professor Jean-Hugues Trouvin.

DR. TROUVIN: Thank you, Chairman. I would indeed like to present to the committee the way the question of BSE has been answered in Europe since the early 1990s. This has essentially been done by presenting the European guidelines on minimizing the risk of transmitting the BSE and TSE. After my presentation my colleague, Dr. Dobbelaer, will expand

and apply the EU approach to the vaccine. Next slide, please.

In this presentation I would like essentially to present the guidelines, the three scientific criteria which are used, some additional measures, discuss also some aspects of the guideline, and then the concrete. Next slide.

The European guideline was issued first in 1991 at the very beginning of the epidemic in Great Britain. The revision of the guideline took place at several locations, but the most significant revision was in 1999 when the guideline became an essential part of the EU regulation regarding TSE requirements in medicinal products.

Originally dedicated to cover strictly the BSE question, the scope of the guideline has been now adapted to cover all TSEs in animal species, particularly ruminants. However, this guideline does not cover the human form of spongiform encephalopathy, and thus excludes products of human origin. For this presentation I will refer only to the BSE aspect.

The guideline covers all types of material derived from ruminants which can be used in medicinal products either as active substances or excipients or even in-process reagents during the manufacturing

process. In other words, all the necessary measures to minimize the risk of TSE in products entering the pharmaceutical industry should apply, whatever the use of the product. Next slide.

As stated in the guideline, the aim of the document is to clearly identify the necessary information for assessing the TSE risk for a given product. Basically, three pieces of information should be considered: The origin of the animals (geographical parameter); the nature of the tissue collected and used; and the products and process or processes.

This clearly shows at the very beginning of the guideline that the risk evaluation is and should be a multi-parameter approach which also takes into consideration the nature and use of the final product. Next slide.

Let's now go to these three criteria. The first criterion deals with sourcing. It is well acknowledged in the guideline that this is the most important criterion. This criterion, obviously, is directly linked with the status of the country regarding BSE cases, and the guideline envisages, thus, three situations and states that the most satisfactory source is from countries which have no

reported cases of BSE. However, the guideline does envisage the situation where materials can be sourced from countries where cases of BSE have occurred.

In this case, additional safety criteria have to be in place in these countries. Needless to say that sourcing from countries where there is a high incidence of BSE should not be envisaged. This recommendation has been made in 1991 and, obviously, is valid essentially for the risk for the beginning of the outbreak of BSE in the U.K.

The guideline envisages also the possibility to make use of well monitored herd, whatever it is located and wherever it is located. As we got now the so called BSE status for a given country, it is important to mention that the OIE criteria are those recommended and used in the guideline.

The BSE status is a very difficult matter, as essentially it does not only rely on the number of cases reported or detected in a given country, but takes also into consideration many other parameters and other risk factors. Next slide.

The second criterion to be taken into consideration is the nature of the tissue collected. As the committee knows, it is necessary to consider

the tissue distribution of infectivity. Depending of the tissue, the risk of collecting an infectious material is thus largely different.

It is important for that to remind the WHO classification -- next slide -- which envisages four categories of tissues, depending on the level of infectivity. This has already been mentioned by Dr. Egan and Dr. Wells.

First -- There are two points on this slide to be considered. The first point is that most, if not all, the bovine derived materials we are dealing with today are classified in Category III, i.e., with low infectivity, or in Category IV where no infectivity is detectable, at least with the limits of detection of the test.

The second point in this slide is that specifically for BSE this classification is a worst case scenario as, in fact, as already mentioned, infectivity distribution in affected cattle seem to be restricted essentially to the central nervous system and some part of the digestive tract. Next slide.

Another point to be considered for the tissue aspect deals with the risk of cross-contamination during collection of the considered tissue. Cross-contamination is a well known source of

risk which, obviously, has to be carefully checked in the collection procedures. Next slide.

Another criterion which has also to be taken on board when assessing the risk is the age of animals. Infectivity, as you know, replicates and accumulates in certain tissues, and it is thus logic, as already shown, to collect from as young animal as possible.

This criteria is, unfortunately, not always applicable, depending on the type of tissue you wish to collect.

In summary for this tissue aspect, it is important to note that, even if the source is in a BSE country, the risk of collecting infected tissues is, obviously, depending on several other parameters and factors such as the tissue itself, the age of animal, and the risk of contamination, not only based on the geographical origin. Next slide.

The third criterion to be considered deals with the manufacturing process. In fact, this notion of manufacturing process encompasses two aspects, the manufacturing process from which the concerned bovine derived material is obtained and the manufacturing process in which the concerned material is used.

For the process which give rise to the

concerned bovine derived material, it is important to mention that some processes can be very "soft," e.g., the collection and processing of the fetal calf serum can be very "harsh."

This is the case for, for example, tallow derivative. I know this is not the subject of today, or even the gelatin, but clearly this process has to be taken into consideration in the risk assessment.

The second process we have also to consider is the manufacturing process in which the material is used. It is also important to mention this process, as it can provide further safety measures, for example, via dilution, via partitioning, and so on.

This is essentially true for bovine derived materials which are used in the production of vaccine, and this point will be illustrated by Dr. Dobbelaer. The guideline, however, does recognize the spatial resistance of the agent to the inactivation process, which is clearly a limiting factor in the safety of those products.

Having gone through the criteria which have to be considered in the BSE risk assessment, I would now like to discuss some of them before concluding this presentation. Next slide.

This slide just to sum up the three criteria which should be considered in the BSE risk assessment. Again, and clearly, this is a combination of factors which contribute to the safety of the final product. Next slide.

I think it's necessary to take a few minutes, if possible, to discuss the geographical criteria. As already mentioned, the BSE status for a given country is often based on the incidence of clinical cases declared or detected in this country. However, it is necessary to acknowledge that incidence is largely depending on the quality of the surveillance system and also depending on many other risk factors in the concerned country, as illustrated in the OID criteria.

There is essentially -- As already mentioned by Dr. Asher, there is in Europe a new proposal to introduce a concept of geographical BSE GBR. From this concept -- next slide -- it is possible to classify countries according to their risk of having BSE cases diagnosed on their territory.

Without entering into detail of this classification, it is worth noting that USA and Canada, as already mentioned, would be considered as being in Class II, and most of the European countries

are considered in Class III. This is to say that the geographical criterion is essentially fluctuating, and the number of cases declared should not be considered as an absolute proof of safety.

What would be the confusion for a given product declared safe because sourced in a country where no BSE cases have been reported if the day after the BSE status changes with one or more cases declared? Should the change in the BSE status be a significant and sufficient reason to consider that the safety of the concerned product is no longer guaranteed? Next slide.

In addition to that, and even if we consider the BSE status as of paramount importance, are the technical problems such as traceability and certificate of origin should also be mentioned. So, clearly, it seems to be reasonable to conclude for this geographical criterion, as explained in the EU guideline, that the geographical criterion cannot be considered as the only safety criterion. It is a necessary criteria, but certainly not an absolute yes or no and sufficient criteria. Next slide.

The geographical criterion is only one of the parameters to be considered. The EU guideline proposes, in fact, a multi-parameter evaluation where

each criterion contributes to the overall safety assessment. Once again, no single approach alone will necessarily establish the safety of the product. This is a combined approach which is necessary in the risk assessment. Next slide.

In this multi-parameter approach other factors should also be considered in the risk assessment, such as quantity, route of administration, etcetera. Next slide.

The guideline is also encouraging the manufacturer to try to get rid of the use of such animal derived material. This is an easily understandable recommendation. However, this recommendation today should not be considered as being in conflict with the current situation where some products are still making use of bovine derived material.

This recommendation is essentially applicable at the development stage of a new product, and we have to acknowledge the current situation that, in some cases, bovine derived materials may still be required because of their special characteristics, as will be explained by Dr. Döbbelaer for vaccine. But this also for many other biological products and even recombinant processes.

Another point which should be mentioned, the last point in the guideline, deals with the quality assurance system. This concept of traceability is also applicable at any stage of the process and contributes to the confidence in the final safety of the product.

In conclusion -- next slide -- Chairman, it's important to remind that, based on the criteria laid down in the EU guideline, all concerned medicinal products, including vaccines, have been reviewed and judged satisfactory in Europe. This review has been also a good experience to show that the risk assessment should indeed take into consideration a number of factors, and should not be restricted to the geographical origin alone.

Finally, this multi-parameter approach is necessary, particularly if one considered the possible evolution of the TSE status worldwide, which makes the geographical origin a very fragile and critical criterion. Thank you for your attention.

CHAIRMAN BROWN: Thank you, Dr. Trouvin. Dr. Dobbelaer has the second part of this presentation.

DR. DOBBELAER: Thank you, Mr. Chairman. Through you, Mr. Chairman, I would like to thank the

organizers to allow me to be part of this decision making, very important decision making which, in my opinion, will -- and it has not been said explicitly today -- will have not only an impact on the U.S. situation but also on the situation in the European Union, and not only in these two continents, but even worldwide, since the very same vaccines and the very same substances we are discussing today are used in worldwide in vaccines.

What I would like together with you is very quickly go over the processes which are used to make bacterial and viral vaccines. I will not dwell very long on that, since it will also be the subject in a more detailed way by William Vann and Ira Berkower.

Then I will emphasize from these production processes what are the quantities of substances of ruminant origin which are to be expected in the final product, and I will also give you a very brief and, I admit, incomplete overview of the different substances of ruminant origin which may be used in production of vaccines, to then come to a conclusion and present the European position which is, in fact, the result of the assessment, an ongoing assessment of individual products and which has

recently been consolidated in a general CPMP commission survey. If I could have the next slide.

This is just to remember the audience that vaccines may consist of either bacterial cells -- the examples here are live oral typhoid vaccine, inactivated whole cell pertussis vaccine -- may contain purified bacterial cell products like diphtheria, tetanus toxoids, acellular pertussis antigens, recombinant hepatitis B vaccine, Hemophilus b vaccine, or may consist of live purified inactivated viral vaccines produced in mammalian cells, such as measles, mumps, rubella, varicella and inactivated poliomyelitis vaccine. If I can have the next slide.

This is a very simplified diagram which shows the production process of a bacterial vaccine, and colored red are the stages at which substances of ruminant origin may be used.

Just to tell you that one very -- Well, first I have to make a restriction, in that in some cases a material from ruminant origin are also used in later stages of the production, such as Tween 80 during purification and gelatin derivatives as excipients or stabilizers in the final product. But I think I can say safely that the tallow derivatives and the gelatin derivatives are products which can

additionally benefit from their production process to evaluate their safety.

Just to stress the importance, the main issue of this slide is that one quantity of seed material yields, in fact, tens of thousands of vaccine doses, and substances of animal origin used in the initial stages of production are, as stated by Jean-Hugues Trouvin, diluted out, in particular those which are used in the seed lot.

Just to give an example, if you take a 1 ml seed, which is first inoculated into a 20 ml pre-culture which is then transferred to another pre-culture of 20 liters, which is then transferred to a fermenter of 1000 liters, the overall dilution factor of the seed is one over 20 million.

Furthermore, I think it can safely be stated as well that the cells which are used for, for instance, virus vaccine production or bacterial cells, are not known to replicate the agents of BSE.

The next slide summarizes the production of viral vaccines, and essentially it is the same message I wanted to give. As a difference with the bacterial vaccines production, here eukaryotic mammalian and avian cells are needed to support virus growth.

I'm going to mention it here. I didn't mention it for the bacterial vaccines. One very important aspect in the production of bacterial and virus vaccines is the use of seed lots and cell banks which provide a constant and reliable source for vaccine production over many, many years. In fact, many of the cell banks and seed lots have been produced and have been the source material to help ensure production consistency for 20 or 30 years.

Again, also in viral vaccines I made this simplified distinction between the different colors. Also in viral vaccines in later stages some substances of ruminant origin may be used, but the point I wanted to make is that, especially for substances used at the level of seed material, the dilution factor also in the case of viral vaccines may be very large, and the dilution factor for a serum used in a cell bank, for instance, is 10^{-8}, which is 100 million times.

Again, the cells used for vaccine production, the mammalian and avian cells used for vaccine production, exclude neural cells and are not known to support prion growth. Next slide.

This is just to give you an idea of the order of magnitude of residual quantities of substances of ruminant origin which may be found at

the level of the finished product of some vaccines.

Just to give you an idea, in the bacterial culture systems where 1 ml seed would be inoculated, etcetera, just the same reasoning I just gave you in pre-culture and in fermenter culture, the dilution factor would be, as I said, 20 millions. One can expect quantities up to 100 nanograms per single human dose in a bacterial vaccine which would use the substance of ruminant origin during the fermentation stage. Next slide, please.

This is to give you an idea of the substances which are used. Not mentioned are amino acids, for instance, and also I should mention that, as I already did, some of these products may be used at stages which are different from the ones stated in the slide. But the main message is that most of these products are used in the introduction of seed lots and cell banks. These are the very initial stages of vaccine production.

As I probably already staged, products such as lactose and gelatin derivatives may also be used in the formulation of some of the vaccines. Formulation is the very -- well, the last but one stage of vaccine production.

The key message I wanted to give here,

that as has been more convincingly demonstrated in previous talks, none of these substances have demonstrated BSE infectivity in studies from naturally or experimentally infected cattle. Next slide, please.

As has been the case, I think, at CBER-FDA, the evaluation process as far as TSE risk is concerned of dosiers for marketing authorization has been an ongoing process since the very appearance of BSE in the U.K., and it has also been a growing process.

I think I can safely state that all EU authorities and all EU manufacturers, as we believe is the case for all authorities and vaccine manufacturers in the U.S. and on the global level, have always been aware and are concerned with the microbiological quantity of biological medical products in general and vaccines in particular.

I think it is particularly true for the TSE issue and its potential ramifications into the field of biological medicinal products such as vaccines and blood and plasma derivatives. From the very beginning of the BSE epidemic in the U.K. in the later parts of the Eighties, all parties concerned have taken measures to minimize the transmission of

the disease to animals and man.

We believe that in the field of medicinal products in general and vaccines in particular, these measures have been very effective, and applying the scientific principles which are laid down in the EU CPMP guideline which has been explained to you by Jean-Hugues Trouvin, and which is now binding EU legislation, and the scientific principles which are used in the -- applying these in the risk assessment and risk management related to vaccines has indeed minimized the risk to theoretical levels.

I just wanted to finish by stating that EU authorities currently see no benefit in additional measures. Should such measures be imposed by other authorities, EU authorities would feel them not to be associated with -- much more -- sorry -- to be associated with risk perception and not with a real risk.

My final slide is certainly not intending to prove that vaccines cannot transmit BSE or prions in terms of the CJD or variant CJD, but this is just to show that, if you classify the cases of variant CJD by year of birth, then one can safely say that vaccines have not been associated with the VCJD epidemic in the U.K., as all have been vaccinated with

vaccines -- well, some of them at least, long before appearance of BSE.

So I think I'll conclude here. Thank you.

CHAIRMAN BROWN: Thank you very much, Dr. Dobbelaer. What we are going to do now is break for lunch, and we are going to have the two brief bacterial and viral vaccine overviews immediately following lunch.

We are going to pick up probably a good half-hour of time early this afternoon, and I hope to pick up another 15 minutes at lunch, because it should be possible for us to reconvene at 1:30 rather than an hour from now. At 1:30.

Before you go out, Bill Freas will tell you about lunch arrangements.

DR. FREAS: In order for us to get back here at 1:30, there is a table reserved in the restaurant downstairs for the TSE members. You are more than welcome to use that table if you so choose. Hopefully, the service will be a little faster.

Some of the Vaccine Advisory Committee members have ordered box lunches. If you would see Nancy Cherry out in the hallway, she will assist you with your lunch. So we're going different directions at this time. See you at 1:30.

(Whereupon. the foregoing matter went off the record at 12:47 p.m.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

(1:36 p.m.)

CHAIRMAN BROWN: It would be much better if I had one of those little bells that you bang, bang; but if I keep talking for a few minutes, probably everybody else will stop. Could we please have some quiet, and could people take their seats, because we would like to start the afternoon session as close to our late time as possible.

As I mentioned before the lunch break, we will now have two very brief presentations on viral and bacterial vaccines. That will be followed by presentations from the industry for public consumption. We already had a closed meeting at eight o'clock which dealt with any particularly proprietary aspects of the manufacture of vaccine.

So without further ado, we will now hear form Dr. Willie Vann. Could we have some quiet over here on the right, please, the FDA group. Dr. Vann, please.

DR. VANN: Today I will present an overview of the manufacturing and a risk assessment of bacterial vaccines. In order to estimate the potential risk of contamination of bacterial vaccines with the BSE agent, we first considered how bacterial

vaccines were made and where bovine derived material is likely to enter the process.

A generalized scheme of how bacterial vaccines are made is outlined in the first slide. The scheme and the calculations that follow are based on our review of currently licensed bacterial vaccines in the United States.

The production of a bacterial vaccine begins with the preparation of a master seed or reference culture, followed by the preparation of a working seed or working culture. The master seed is a well characterized reference culture from which all bacterial cultures used in the manufacture of a particular vaccine component are derived. Next slide.

The working culture is derived from the master seed, and is stored in aliquots to be used for routine production of batches of vaccine. An aliquot of the working seed is used to generate an inoculum for the fermentation process. Next slide.

The fermentation step is the growth phase where bacterial culture is expanded and produces antigens for vaccine production. The antigen for the vaccines are recovered from the culture, purified and converted into a final container product.

The preparation of an entry of master seed

and working seed cultures in the process involves significant dilution of the culture which can range from 10^{-2} to 10^{-3} for a given step. Next slide.

After review of current practices in the manufacture of bacterial vaccines licensed in the United States, only a few components were identified with the potential for the introduction of BSE agents via bovine derived material. These manufacturing components given in this slide are media components and stabilizers for seed culture storage.

The media components are primarily bovine derived broths. The stabilizer of polygeline is used for the long term storage of the master seed. Next slide.

This slide outlines the points at which bovine derived material would enter the manufacturing process. Thus, the potential for entry of BSE agent would be in either of three places, the master seed culture, the working seed culture, or the fermentation broth.

Because of the small amount of media introduced into the seed culture steps, due to the high dilution, these steps are considered to be less -- have less of a potential risk than at the fermentation step. Approximately one to 10 milligrams

of protein derived culture media is introduced at either of these seed steps prior to fermentation.

The potential risk is increased for the use of bovine derived material in the fermentation broth, because of the large amount of media required for fermentation in a batch. A fermentation broth requires hundreds of grams to several kilograms of media protein. Next slide.

The next important issue to consider is the potential infectivity of the bovine derived material used in the preparation of the media. We have used in our estimation the infectivity categories outlined by the European Union which have been generally adopted for the estimation of the risk of BSE in culture media.

For our risk calculations we have relied on data supplied and published by European Union committees, and on the methods published by Bader, et al., in 1998. These latter methods were the results of deliberations of the BSE Committee of the Pharmaceutical Research and Manufacturers of America.

The European Union system has four categories of infectivity, the most infectious for BSE agent being Category I which includes nervous tissue, and the least being Category IV. The infectivities

given in this table are based on values published by the PhRMA BSE Committee.

In the next slide is given a list of media components -- of media containing bovine derived material used in the manufacture of bacterial vaccines and the EU categories of the tissues used in the manufacture of these media. All of the bovine derived material used for bacterial vaccines are derived from either Category III or Category IV tissues.

Thus, in our estimation we have used a theoretical mixture of Category III and Category IV materials. The assumptions that we used to make our risk estimate are given in the next slide.

First, we do not assume a species barrier. Secondly, the conditions that we are considering is where the bovine tissues are sourced from a country in Europe other than the U.K. Because variations in butchering practice methods for the preparation of bovine muscle tissue could result in contamination with nervous tissue, we have done the calculation for two scenarios.

The first scenario assumes that the skeletal muscle is free of contamination from Category I tissues. The second assumes a .01 percent contamination with Category I nervous tissue. This is

equivalent to approximately a tenth of the spinal cord per cow. This latter scenario provides a worst case based on our current review of manufacturing where a small amount of Category I material could enter the manufacturing process.

We used these assumptions and the information outlined above to calculate a risk assessment based on the method outlined in the Bader article. This method is outlined in the next slide.

The potential risk of an infected animal used in manufacturing is given by the regional risk of an animal -- of an infected adult animal. This value and the number of animals used per batch of media are used to calculate the risk of a batch of vaccine being contaminated by an infected animal.

The infectivity of the bovine tissue is estimated next. This estimate relies on the estimated infectivity of appropriate tissues from an infected cow. The estimations given in the next slide are based on the German quantitative system, since infection with Category III and Category IV bovine materials has never been observed.

The German system uses scrapie by analogy as a model to estimate relative infectivities. Thus, these values could be a likely overestimation of the

infectivity of the bovine tissues. Next slide.

The species barrier in this equation is one, since we do not assume a species barrier. The estimated number of infections per contaminated batch is then calculated by multiplying this value by the correction for the route of administration and the process reduction factor.

The route of administration is intramuscular, which is 100 times less infective than intracerebral, for which the infectivities in the previous table was generated.

The major process reduction step is autoclaving of the bacterial growth media, which is assumed to be tenfold. All media containing bovine derived material are autoclaved prior to use. This autoclaving step and the low level of purification accounts for the reduction of risk due to process. In the last equation we are assuming a batch size of one million doses of vaccine. Next slide.

For our potential risk calculation, we have used two theoretical tissue compositions for calculating human infectivity. Both theoretical mixtures contain Category III and Category IV derived materials.

In scenario one, the skeletal muscle added

to the broth is free of contamination with Category I nervous tissue. In scenario two, it is assumed that the equivalent of one-tenth of a spinal cord contaminates the skeletal muscle from one cow.

Since the largest amount of bovine derived material that could potentially be introduced into the process is in the fermentation broth, we have outlined these calculations using the above method in the next slide for fermentation broth.

In this calculation we used the risk of an infected animal for Europe of 10^{-4} or one in every 10,000 cows. This value is arrived at using the EU scheme by multiplying the incidence in the worst case country by a factor of ten to obtain a potential risk. Since we are assuming that only one cow is used per batch, the risk of a contaminating batch is one times 10^{-4}.

The estimated infectivity is based on our theoretical media composition. The estimated number of infections per contaminated batch is reduced by a process factor of 20, mainly due to autoclaving.

Finally, the risk of a contaminated dose based on use of bovine fermentation broth is 5×10^{-10}. This is one in every 2 billion doses. For scenario two, the potential risk is increased to 10^{-8}.

In the next slide is given an estimate of the risk for the use of bovine broth in master seed or working seed using the same assumptions. In the latter case, the risk is very small, one in every 200 billion doses, even when one assumes a 0.1 percent mixture of Category I nervous tissue with the Category IV material.

The values that we have presented in this assessment are, in our judgment, a realistic worst case scenario. Thank you. Thanks to the other guys who helped me.

CHAIRMAN BROWN: That was, in fact, a conclusion, Dr. Vann?

DR. VANN: That was, in fact, my conclusion.

CHAIRMAN BROWN: Okay. Well, we thank you very much, and we will now move on to a similar parallel discussion or presentation of viral vaccines by Dr. Berkower. Both Dr. Vann and Dr. Berkower are in CBER, which is part of the FDA.

DR. BERKOWER: Today I discuss the potential risk of BSE contamination in viral vaccines. The main risk of BSE contamination comes from bovine material added to the culture medium used for growing cells and virus.

FDA review has determined that fetal calf serum from the United Kingdom has been used to make certain viral vaccines. This fetal calf serum was obtained at a time when the BSE epidemic was just getting underway in the U.K. USDA has estimated the incidence of BSE in adult cattle at that time as about one in 200.

Other data presented today have suggested that maternal-fetal transmission was on the order of ten percent, resulting in one calf in 2,000 becoming infected. Since the fetal calf serum is often pooled in lots typically of the size 1500, it is quite possible that some fetal calf serum from an infected calf could be included in the pool used for vaccine production. Next slide.

This slide shows typical steps in vaccine production where bovine material is used. Vaccine production proceeds along two paths, here and here, which converge at the bottom, resulting in production of the vaccine. On the right, virus from the original isolate is expanded first to the master seed and then to the working seed where it is ready to be used in the production.

At each step in this expansion fetal calf serum and cells are used to grow the virus, here and

here. Thus, if the calf serum or the cells are contaminated, they could contaminate the growing virus.

On the left side cells are grown from the cell source up to a large scale, and then frozen to make the working cell bank. Typically, the working cell bank may include as many as 1,000 vials of cells. At the start of a production run, a manufacturer would thaw one vial and expand that to a bioreactor size infected with the virus and produce viral antigen, which is then subject to pretty limited steps of purification and/or inactivation to produce the final vaccine.

As shown on this slide, fetal calf serum from the U.K. has entered the production process at steps marked one and two in red. So U.K. fetal calf serum was used to produce the working cell bank. About 5 mls of U.K. fetal calf serum were used to make each vial of the working cell bank. These cells are then -- The one vial is then used to make a production lot, which is typically on the order of 500,000 doses of vaccine. So 5 mls makes 500,000 doses.

The question is: If these 5 mls of fetal calf serum became contaminated with the BSE agent, what would be the risk of BSE coming through to the

final product? Note then that each ml of fetal calf serum at the working cell bank is used to make 100,000 doses of vaccine.

In order to calculate this risk -- next slide -- we made certain assumptions about BSE. First, we assumed that one in 2,000 fetal calves was infected. Second, we assumed that each ml of fetal calf serum from this calf contained approximately less than one infectious dose of BSE.

This estimate is based on partially completed experiments in which cell concentrates made from cow blood -- that is, buffy coats -- were shown to be noninfectious cow to cow. Noninfectivity of 1 ml could certainly represent less than one infectious dose per ml or it could actually be less than one infectious dose per ml, but we have conservatively assumed less than one.

Third, we assumed that the number of BSE agents introduced at the top of the process I just showed you on the previous slide would equal the number that came through into the vaccine. Thus, the risk would be the input number of BSE agents divided over the number of doses given.

We have allowed no risk reduction for purification, because in many cases the purification

is minimal, or for species barrier which is basically unknown in the case of BSE from cow to man. We have, however, allowed a factor of 200 for reduced transmission by the intramuscular route.

Given these assumptions -- next slide -- we can calculate the risk of BSE getting into vaccines as the product of four separate risk factors shown on this slide.

The first is we assumed less than one infectious unit per ml of fetal calf serum. The second was we know that the infectious unit is diluted into a pool of 2,000 normals. Third, we know that the cells from one ml of fetal calf serum make approximately 100,000 doses of vaccine in a typical production scheme, giving us a factor of 10^{-5}. Fourth, we have allowed a 200-fold reduction for the route of administration. Those are our four factors.

Multiplying those four together gives us a cumulative risk of infection per dose as $.25 \times 10^{-10}$. This means one BSE infectious dose per 40 billion vaccine doses.

Now each of our assumptions comes with its own uncertainty, and some of these would be rather large. Next slide.

First, we assumed the incidence of BSE in

cows to be one in 200 in the early years of the epidemic in the U.K. That is, in the mid-1980s. This is based on estimates of the USDA.

Second, we've assumed that transmission from the mother to fetal calf was about ten percent, based on the study of Wilesmith presented earlier, although others have challenged this estimate.

Third, the infectivity of fetal calf serum from an infected calf may be significantly less than one per ml, as I said, because the experiments have basically shown that transmission was not detected when approximately 1 ml was used in a sensitive bioassay.

Fourth, there may be additional risk reduction factors which we have not allowed for, such as a species barrier between cow and man.

Fifth, partial purification of vaccine may contribute a little more. We have allowed no reduction for purification, because the purification scheme was not designed to remove BSE agent and has never been shown to remove it.

Finally, our overall estimate obtained by multiplying values with large errors could itself vary over a very large range.

In summary -- the next slide -- we

estimate the risk of BSE transmission as less than one BSE infectious dose in 40 billion vaccine doses for viral vaccines made with U.K. fetal calf serum in the mid-1980s.

CHAIRMAN BROWN: Thank you, Dr. Berkower. Question? Yes?

DR, GRIFFIN: It seems to me, especially for the virus vaccines, the biggest assumption that we're making wasn't on your list, and that's that there is no evidence that the agent can replicate at all in the cells that are being used to produce the vaccine.

I'd just like to know what kind of data we have for that assumption.

DR. BERKOWER: Well, there has been experience in getting BSE agent to replicate -- or I should say, TSE agents to replicate in cell culture. These have worked entirely on cells of neural origin, such as neural blastomas. They have not worked on cells that would typically be used for vaccine production.

DR. GRIFFIN: How hard have people tried? In general, you get a system that works. That's what you work with, but going to the apposite or, you know, can you really not do it in another kind of cell is a

different problem.

DR. BERKOWER: There are a number of things that could be done that have not been done to assess the ability of TSE agents to grow on the cells that are used for viral vaccine production. For example, they could simply try and infect the actual cells that were used or they could measure the PrP status of the cells that were used at the end of a fermenter run or -- There are many other things that could be done. Those are two that I would like to see.

As Dr. Egan said at the start of this, there are many factors that we would like to know which we don't know at this time.

CHAIRMAN BROWN: The answer is correct. We did in our own laboratory many, many years ago all kinds of cell culture efforts with a number of human -- not BSE but a number of human TSEs, and they just diluted out over a few passages. They simply don't replicate in cell cultures typically.

DR. GRIFFIN: Right. Now I appreciate that, but I think that's still a little bit different question than could they possibly, and does BSE, because it does cross species barriers a little more easily than some of the other TSEs, in my

understanding.

CHAIRMAN BROWN: Well, I don't know if that's true. It certainly crossed the human species barrier.

DR. GRIFFIN: Well, that's the one we are most worried about.

CHAIRMAN BROWN: The most important, yes. Exactly. So if we're using, say, human cells, it may be that they are more facilitated by that than, for example, a strain of scrapie. I think maybe that's what you're talking about.

DR. GRIFFIN: Exactly. And we know that they go into non-human primates as well, and those are frequently the source of the kinds of cells that are used in viral vaccines.

CHAIRMAN BROWN: Is Dr. Sue Priola in the room? I understand that she might have some information about cell cultures. Please.

DR. PRIOLA: Well, I don't think I can add much to what Dr. Brown said, but historically the only cells that have been susceptible are either neuronal in nature or fiberglass. There's been reports of fiberglass.

Those experiments have all been done with rodent models of scrapie. There's only one instance

that I can recall of sheep infectivity being passed into culture. Other than that, no work has been done with BSE that I'm aware of.

So in addition to the difficulty of getting the agent into the cells, it's very difficult to maintain it. As Dr. Brown said, you tend to lose it very quickly and, when it does get in, it's at very low levels.

CO-CHAIRMAN GREENBERG: I would just add that this experiment is different yet again, because it's a co-cultivation with a virus. Nobody in the right mind would do that experiment in their laboratory, but the virus could facilitate as well as interfere with transmission in cell culture.

In each case, it would be a different virus. So it would be complicated to figure it out.

DR. GRIFFIN: Right. But you could do some kinds of experiments, like even co-cult with a neuroblastoma cell line or something that you knew was very susceptible or the most susceptible to your agent of interest.

CHAIRMAN BROWN: Ray Bradley or any representatives from the United Kingdom, are you aware of any effort, any attempt, anything that is going on right now with respect to attempting to grow

specifically the BSE agent in any cell culture?

DR. BRADLEY: Nothing that I'm aware of in the master program at all.

CHAIRMAN BROWN: Okay.

DR. BRADLEY: If I could just comment briefly on the bioassay in cattle from tissues from cattle of buffy coat, you suggested that those studies were completed.

DR. BERKOWER: I think I said incomplete.

DR. BRADLEY: Sorry.

DR. BERKOWER: I said partially complete. That's what I said. I have it right here.

DR. WELLS: If I could just give some detail on that partial completion, basically, we have assays from the pathogenesis study at six months post-exposure, 18 months, 26 months, and 32 months. Only the material from the 32 months has reached 43 months p.i. which, you know, might be assumed to be a reasonable incubation period, if it was going to -- if disease was going to occur.

The others are all below 20 months, which would be somewhere -- unless we're going to say that there is above 10^3 cattle units in there, then we can't draw conclusions from that.

DR. BERKOWER: Okay. I'd like to just

repeat what you said. If you do assays in mice where there's a species barrier of 10^3 and you can't even inject a ml, but let's say you did because you did a lot of mice, you could say it was less than 1,000 per ml. That's the best you could say. That's what you just said.

Also I was aware of the buffy coat from the animals when they first lit up the brain in your pathology study, pathogenesis study, being used; and I know they are a little under four years and that the animals are okay at this point. I believe it's four cows that it's been assayed in.

DR. WELLS: That's right.

DR. BERKOWER: Four cows. Yes, and the way I calculated the less than one per ml is that a buffy coat is roughly a tenfold concentrate, that to use it in the brain it was diluted roughly tenfold an the volume injected in the brain, obviously, was about one ml. So it's about one ml worth of cells, which would be roughly equivalent, if this were scrapie and if it was Dr. Brown's experiments, to one ml of serum.

DR. PRIOLA: Dr. Brown, may I make one more brief comment?

CHAIRMAN BROWN: Yes. Sure.

DR. PRIOLA: When these infections are

successful in tissue culture, it usually takes an extremely high level of infectivity to get it to go, that usually they are very inefficient. At the lower multiplicity of infection you go, the less efficient the process. So in most cases, you have to start out with quite high levels.

CHAIRMAN BROWN: Dr. Almond, you had your hand up a minute ago.

DR. MODLIN: Actually, I wanted to pursue exactly the same line of questioning that Diane just did, and I think I had just about all my questions answered except just to point out that in this system the fermentation in cell culture for a virus, presumably polio virus or whatever, is going to be very short. It's going to be a matter of just a few days, and here we're talking about an agent that requires presumably an incubation period of much, much longer than that, even in cell culture, to detect it.

I guess that was a question. How long does it take -- How long do you have to maintain it in cell culture before you can detect it in your most sensitive system?

CHAIRMAN BROWN: Well, what typically happens is, if you inoculate it progressively over a matter of one or two or three passages, it disappears

rather than replicates, and the question -- the reverse question, how long does a successful take require, I will defer to you.

DR. PRIOLA: You can detect the protease resistant form of the prion protein in our hands after a day, if you overlay the homogenate. But to get a successful infection, you usually have to wait 30 days to ensure that it's replicating, but if it's present at the first pass, it may be present at -- you know, on pass three and pass five. It might be present continuously or it might be lost by passage two. It's unpredictable.

CHAIRMAN BROWN: So like everything else, the answer helps a great deal.

DR. GRIFFIN: Well, but the point is that that's how long it takes for you to detect it. But if the infection is successful, it's successful on the first few hours. I mean, you know -- I mean, it's there or it's not there, and then how long it takes to build up to the point of detectability in whatever assay you are using is another issue.

DR. BERKOWER: Can I just say one thing on this? So in our calculations we had a little thing we called prion equals prion-out or BSE-out. We didn't assume that it grew, and we didn't assume that it just

disappeared. We assumed that it hung onto the cells, was not washed off during the typical incubation period, and that's sort of -- I think that's about what everyone has said.

CHAIRMAN BROWN: Dr. Almond, last comment on this?

DR. ALMOND: Just to echo your comments, Mr. Chairman. In my laboratory we also tried very hard to establish prions in cell culture, and we worked, in fact, with Sue Priola on some aspects of that. We didn't manage it. But the point I wanted to make was that a lot of cell cultures that have been kept and maintained in U.K. laboratories over the last 20 years will inevitably have used U.K. sera.

I don't know of any evidence that when you look at those cells, you detect any PrPSE in any of them. In fact, it's very hard to detect PrP at all in those cells. I think that probably points to the fact that it really is not easy to infect these cells with prions.

Chris Berkett is probably the person at the U.K. program that's most experienced working with these so called SMB cells, which were derived from a scrapie mouse brain back in the 1970s. He has managed to do some work with those cells, but I spoke to him

recently, and it's clear that he also has great difficulty in infecting cells.

CHAIRMAN BROWN: And you have the other problem, that PrP detection is typically infinitely less sensitive than a bioassay. So what you would really be obliged to do would be to bioassay all your cell systems. Dr. Roos?

DR. ROOS: I just wondered how common it might be that one contaminated the fetal calf serum during its collection, for example, with instruments that might have been used for slaughter and contact with central nervous system tissue, especially when we go back to the early years in the BSE epidemic.

CHAIRMAN BROWN: Ray Bradley, could you make a comment with respect to the potential risk of cross-contamination in a slaughterhouse during the period in question, 1980 to '95?

DR. BRADLEY: Well, I can only, Mr. Chairman, answer the basic information. When one collects fetal calf serum, if one was collecting it, of course, the cow is already dead. The uterus is removed, and then the fetus from that uterus is taken away, and there would be no possibility of cross-contamination, I think, with central nervous tissue in the normal method that people would utilize to collect

this.

Of course, the qualities of collection, going back very historically, could not be necessarily claimed to be the same as they are today, but I haven't got any possibility of commenting on that, never having done it. But I think actual cross-contamination with central nervous tissue would be most improbable, even central nervous system from the calf, because this would not be opened or touched in any way.

CHAIRMAN BROWN: Is that okay, Ray?

DR. ROOS: I assume that the instruments that might have been used on the central nervous system of the mother is also perhaps used in the collection of the fetal calf serum. Am I correct?

DR. BRADLEY: I'm sorry. I just missed the first part of your question.

DR. ROOS: I'm assuming that the instruments used to collect that fetal calf serum might be the same that could have been contaminated with the central nervous system of the mother.

DR. BRADLEY: No, certainly not. I would think that would be most unlikely. Well, it would be, I think, not possible, certainly in the modern ear, because the --

DR. ROOS: No, I meant --

DR. BRADLEY: Historically?

DR. ROOS: Yes.

DR. BRADLEY: I do not think that there would be any practical connection between those instruments. I mean, we are talking here about trying to collect a sterile product, forgetting all about BSE. Those who are involved in this procedure do take very, very careful precautions to prevent any form of cross-contamination, whether it be bacterial, environmental, viral or anything.

The methodologies used now involve sterile equipment, disposable equipment, cardiac puncture, in an environment which is essentially divorced from the central nervous tissue in the slaughter hall. This would not be permitted to be done in the slaughter hall. So there would be no connection. They would be different personnel trained for different purposes.

DR. ROOS: I don't want to belabor this, but I want to go back 15 years ago. It just wasn't clear to me. So the instrument that is used to collect the fetal calf serum would not have been used with respect to the mother and the mother's tissue. Is that what you're saying? Totally different --

DR. BRADLEY: Exactly.

DR. ROOS: -- sterile, newly packaged scalpel?

DR. BRADLEY: Exactly. In order to --

CHAIRMAN BROWN: Yes, that's what he said.

DR. BRADLEY: In order to collect the blood, you would use a needle. A needle would not be involved at any point in the slaughter. So just from practical common sense, it wouldn't be done. But I cannot speak from personal knowledge of that procedure historically.

CHAIRMAN BROWN: Bearing in mind, of course, that a slaughterhouse is not a P3 facility.

Thank you. We now have presentations from two manufacturers of vaccines, and the first will be from SmithKline Beecham Pharmaceuticals, and Dr. Clare Kahn will introduce the subject, which will be followed by a presentation from a gentleman who you now are familiar with, Dr. Ray Bradley.

DR. KAHN: Good afternoon, members of the committees, ladies and gentlemen. I'm Clare Kahn, and Vice President, North American Regulatory Affairs, responsible for vaccines and representing SmithKline Beecham.

We have a generic presentation for you today, and to deliver this it's my pleasure to

introduce to you Dr. Ray Bradley, CBE. He will broadly review the topic of TSE risk from bovine derived materials, making special reference to all of the considerations for their use in vaccine manufacture as raised by the agency for today's discussion.

Dr. Bradley served as head of the pathology at the Central Veterinary Laboratory, now called the Veterinary and Laboratory Agency, in the U.K.'s Ministry of Agriculture, Fisheries and Food, or MAFF, from 1983 to 1991. These were seminal years in the history of BSE.

During this time BSE was discovered. Dr. Bradley initiated and collaborated the initial BSE research program, and he was heavily involved in national and international issues for BSE and other animal TSEs.

Dr. Bradley served as the BSE coordinator for MAFF from '91 to '95, and since that time he has served as an independent BSE consultant to WHO, OIE, EC, U.K., and the Argentine and the U.S. governments' committees and expert committees and expert consultation.

In his consultancy with SBE, Dr. Bradley has conducted a comprehensive review of BSE risk from

bovine derived materials, and also review of the provenance and TSE risk in starting materials of bovine origin used by SBE in vaccine production for worldwide markets.

So now it's my pleasure to call upon Dr. Bradley to present to you on the TSE risk from source materials derived from cattle and used in the manufacture of vaccines for human use.

DR. BRADLEY: Mr. Chairman, members of the committees, ladies and gentlemen, good afternoon or, if you're from Europe, good evening. If I could have the next slide, please.

The objectives of my talk are to discuss the TSE risk from source materials derived from cattle used in the preparation of master working seeds and cell banks, fermentation processes, and in the formulation of final products.

First of all, just a recapitulation and reminder of where BSE occurs. The red countries are those with cases in native-born cattle, and those in blue are countries with BSE in only imported cattle. Next slide, please.

Here is a reminder of the epidemic curve for the U.K. based on annual report cases, and I refer to the large number of BSE cases in the U.K., 176,000.

By comparison, throughout the rest of the world the total number is some 1300. Next slide, please.

Once you have BSE in a country, there are possibilities to export it in incubating healthy live cattle. That has been done by accident, of course, from the U.K. to various countries which are quite widely dispersed, as you see, but very small numbers of animals which in themselves present very low risk, provided they are detected and destroyed. Next slide, please.

However, it is not just cattle which actually present the risk. The other risk comes from the export of meat and bonemeal contaminated with BSE material. As you can see, from the U.K. quite large quantities and meat and bonemeal were exported for the European Union. Even a small amount was exported to the North America, just 12.3 tons, in 1984-85, which was, of course, a risk period.

From other countries with BSE, there's a lot less certainty as to how many cattle and how much meat and bonemeal might have been exported elsewhere. Next slide, please.

The next point I want to make is the importance of the factors governing transmission of transmissible spongiform encephalopathies. There are

three factors, the dose, the route of exposure and the species barrier, which clearly comes into play once you cross a barrier between two different species.

The dose is the mass or volume multiplied by the titer in unit mass. In regard to the route of exposure, there are widely differing efficiencies of different routes. Most of the talks that you've had today and much of mine will be referring to transmissions done by the intracerebral route, which is the most efficient of all.

The oral route is the least efficient, and the intramuscular route is rather closer to this end of the scale than it is to this one. Next slide, please. Sorry, could I just go back? The species barrier is determined by two features or two factors, firstly the strain of the agent and, secondly, the variation in the PrP gene sequence between the donor and recipient species. Next slide, please.

So the summary from the geographical risk from cattle with TSE: We can say that the risk could be derived from two sources, exogenous sources and endogenous sources. Exogenous sources include importation of infected cattle or meat and bonemeal, and endogenous sources means genesis of TSE in cattle from any species and recycling it via the feed in meat

and bonemeal.

To conclude on this slide, the precise geographical destination of cattle and meat and bonemeal exported from all countries with BSE is uncertain. Thus, the analysis of risk of TSE infectivity by type of tissue is of fundamental importance. Next slide, please.

So that's what I want to pass to now. As you have heard from Mr. Wells, in natural cases of BSE there is a very restricted distribution of agent infectivity to the central nervous tissue and included in the retina and spinal cord and brain. Next slide, please.

However, when one challenges mice by the efficient intracerebral route, including the intraperitoneal route at the same time, we find a whole range of tissues in which no detectable infectivity can be found. They are listed here. Mr. Wells mentioned 51 tissues on this slide.

I draw attention to those in blue, which I'm going to talk about in a little more detail, because these are tissues which are used as starting materials for manufacture of vaccines. I will also draw attention to the negative transmission studies from a series of male and female reproductive tissues

and including fetal calf blood. Next slide, please, and the next.

Mr. Wells has elegantly explained his pathogenesis study to you, and this is a summary slide giving the essential data from which I wish to draw one or two clear points.

First, that only the distal ileum shows infectivity at early stages of incubation. Second, in the other tissues marked in red, which do show infectivity, none of them show infectivity more than three months before the clinical onset of disease. So this window period that's been mentioned is very small. Finally, a whole range of tissues, which I'll deal with in a little more detail later, show no detectable infectivity after bioassay in mice at any stage of either preclinical or clinical disease. Next slide, please.

The specific items I wish to consider are listed here, and I'll deal with them in turn. Independent judgment that milk is safe after consideration of the results of transmission and epidemiological studies have been determined by the WHO, the OIE, the EC, and the U.K. SEAC. They have evaluated the data and concluded, in a nutshell, that milk is safe. The USDA has no restrictions on the

importation of milk, presumably coming to the same conclusion. Next, please.

Now to summarize the results from all the collective studies that have been done on blood. First of all, in natural BSE in cattle in this column and then in experimental BSE in cattle in the second column, I've listed here not just blood itself but other tissues which are closely associated with blood such as spleen, lymph nodes and bone marrow.

So in natural BSE, not one of these tissues has shown any detectable infectivity when tested in mice, and of the two that have already been tested in cattle, Mr. Wells reported this morning by intracerebral inoculation, the most efficient route, no detectable infectivity there either.

When we come to the experimental BSE, the pathogenesis study, again buffy coat was bioassayed. It shows no detectable infectivity. Likewise for these other tissues during incubation, including the bone marrow during incubation, and the buffy coat has bee bioassayed in cattle, although that has not yet been completed. It has so far gone for three and a half years, as you see here, and the animals are still alive and healthy, giving us confidence but not absolute, complete reassurance at this point in time.

Next slide, please.

I now pass to muscle and pancreas. To summarize these two issues which have been part of the concern, skeletal muscle and pancreas from cattle affected with natural BSE have shown no detectable infectivity after bioassay in susceptible mice. Furthermore, BSE infectivity has not been detected in skeletal muscle or pancreas at any stage of incubation of experimental BSE, also following bioassay in susceptible mice. Next, please.

Now passing to derivatives of gelatin which, as source material, start from bovine bone, and I'm speaking here specifically about polygeline. There is no detectable inherent infectivity in the raw material from clinically healthy animals that is, in bones. However, the TSE risks in bones historically used for gelatin manufacture may not have been negligible due to the contamination or possible contamination with central nervous system tissue. But -- and this is an important "but" -- the process in producing polygeline involves an important clearance factor of many logs of loss of infectivity. Next slide, please.

So the conclusions from this are that no BSE infectivity has been detected in skeletal muscle,

pancreas, spleen, blood or any component of blood of cattle or bovine fetuses in natural or experimental BSE or in the milk in natural BSE. There is no epidemiological evidence that bovine milk, blood or any blood component carries BSE infectivity.

Here is an important point to stress. BSE is different from scrapie. In the early days of this epidemic, we were less certain about that. We are now sure that it is not the same as scrapie, and we cannot use the data for scrapie to make the risk assessments if we have new data generated from the species and tissues in question.

The WHO and CPMP classifications based on observations of scrapie in sheep and goats showing low infectivity Category III for pancreas and medium infectivity Category II for spleen are, therefore, not applicable to cattle potentially or actually infected with the BSE agent. Next slide, please.

I now want to come to the more concluding part of my talk in regard to possible <u>in utero</u> maternal transmission of BSE. Next.

I think it's very important indeed for you, particularly if you are not veterinarians, to understand what we mean by maternal transmission. It means transmission from dam to offspring at one of

three stages, in utero -- and this is the point at which fetal calf serum would be sourced.

So the subsequent other two forms of maternal transmission such as infectivity getting to the fetus during parturition or in the immediate post-parturient period do not count. This is the only one that has to be proved to show if there was an infectivity here, and I shall try to demonstrate there is not. Next, please.

In regard to maternal transmission, the cohort study and the case controlled study that Mr. Wilesmith mentioned this morning did not address the question of occurrence of infectivity in fetal calf blood. That was not a defect of the design. It was never intended, and so it could not report on that feature.

Neither study demonstrated the existence of maternal transmission in the absence of a feed-borne source, a very important feature. Neither study demonstrated the occurrence of in utero maternal transmission, the only one which could potentially incriminate any risk factor in fetal calf serum. Next slide, please.

In regard to the general points about maternal transmission of BSE, in the U.K. in no case

has the observed annual incidence of BSE in offspring of confirmed cases exceeded the expected incidence from the feed-borne source alone. In the EU outside of the U.K., I've mentioned that we have had outside there 1283 cases of confirmed BSE. No case of BSE has been reported in the offspring of a case. So that figure is zero.

In Switzerland, an even more thorough study was done. Brains from 182 offspring of BSE cases have been examined microscopically and for the presence of prion protein. No evidence of BSE was found in any case. Next slide.

So the summary on this: No infectivity has been found in any reproductive tissue, whether male or female. Cohort and case controlled study are not designed to determine infectivity in fetal calf serum. No studies have demonstrated maternal transmission in the absence of a feed-borne source, and the results of these studies do not contradict any of the evidence supporting the absence of detectable BSE infectivity in fetal calf serum. Next, please.

My last slide and conclusion is, therefore, that the assessment of TSE risk in the starting materials of ruminant origin that are used for the manufacture of vaccines has revealed no

evidence for a degree of risk that is higher than negligible. Thank you.

CHAIRMAN BROWN: Thanks very much, Dr. Bradley.

DR. KOHL: I think it's a very important presentation, and I'd like to verify or possibly challenge a couple of points.

Clarify for me the bone marrow experience. We were told this morning, I believe, that several bone marrows were positive during, I think, the latest stage of BSE.

DR. BRADLEY: I'm just going to put the slide up which will answer your question. This is the pathogenesis study. Let me wait for the slide. And bone marrow is listed here, and you see that the bone marrow is a singleton positive result which occurred in the clinical phase of disease from which we do not collect, and there is a paper written by Mr. Wells and his colleagues giving the possible explanations for this.

I wouldn't wish to go into the detail on that at the moment, but if that's helpful to you.

DR. KOHL: That is. On your other slide, the bone marrow was referring to during incubation. Is that correct?

DR. BRADLEY: I'm sorry?

DR. KOHL: Your other slide which said bone marrows were negative is referring to the incubation period?

DR. BRADLEY: Yes. This one, there's no infectivity in incubation, no case --

CHAIRMAN BROWN: Do you have a follow-up? That's okay, Ray. Go ahead.

DR. KOHL: Now on the cohort study which was presented this morning there was a ten percent risk of -- I'm not going to say transmission, but of BSE in offspring of infected cows. In that study, as reported in our briefing document, the relative risk was 3.4 percent -- 3.4 relative risk between offspring of infected cows compared to offspring of non-infected cows.

DR. BRADLEY; Yes.

DR. KOHL: Now you've told us and everybody else has told us that milk is not infectious. Is that correct?

DR. BRADLEY: Yes, no detectable infectivity in bovine milk.

DR. KOHL: Okay. So we can disregard the infectivity from mother to child or to infant by milk.

DR. BRADLEY: No.

DR. KOHL: Well, either it is or it isn't.

DR. BRADLEY: That's not -- You are assuming that could be the only post-natal origin.

DR. KOHL: I'm just talking about milk. Milk is not infectious.

DR. BRADLEY: Okay.

DR. KOHL: Is that right?

DR. BRADLEY: Yes.

DR. KOHL: Okay. And these cows, the infected babies or whatever you call calves, and the non-infected calves -- I'm a pediatrician -- are kept -- My understanding is they are kept on the same farms, fed the same food, and in the same environment. So presumably, the risk of transmission to these calves in the post-partum period is similar.

So I am left with the assumption that either this relative risk of 3.4 is due to intrapartum transmission, in which case the fetal calf serum would not be affected since there is no partum period when you collect fetal calf, or due to in utero transmission.

Now I agree with you that the studies don't prove that it's in utero transmission, but one can definitely not assume in that study -- in fact, in any study so far -- that there is no intrauterine

transmission.

DR. BRADLEY: Well, I think there's several points I should make. The first point, and I think Mr. Wilesmith made it very well, was that he said his study did not demonstrate maternal transmission. It demonstrated there was a maternal factor involved in the different observations that were made in the two groups of animals.

DR. KOHL: Well, I'd like you to describe any maternal factor other than breast milk, intrapartum infection or intrauterine infection. As a pediatrician, my area of expertise is congenital infections, and I've published a bit on that, and I'm not aware of any other maternal factor.

DR. BRADLEY: Okay. In the course of the study, just to indicate -- and Mr. Wilesmith can perhaps chip in if I've got the major fact wrong -- that the calves that we used, the 600 calves, were pairs, of course. But they were not collected at birth from farms and then moved to the environment on the experimental farms in which we looked after them.

So they stayed on their natal farms for some period, sometimes for a year, perhaps longer. So that's the first point, before they were all collected together on the three separate farms, but kept as a

pair.

The calves were collected from three birth cohorts, and during the course of these birth cohorts the risk from BSE from feed diminished. If you analyze the figures in the cohort study, there was an equivalent decline or a similar decline in the incidence of BSE as you got further away from the feed ban which was in place.

Thus, there is an association of a reducing risk with time associated potentially with feed. So let's just see how that maternal factor could operate. It could operate on the basis that some particular animals eat more. So they had a greater opportunity to consume infected feed, if there was any infected feed, and that I'm speaking of on their farm of origin before they came to the Ministry farms. I don't know if that will go any distance to answer your question.

If I may, Larry, could I just ask John to add something to that, because it was his experiment.

DR. WILESMITH: Ill try and clarify this. One of the things that is of interest is that what I did in terms of the design of the study was to take account of this continuing feed-borne risk.

There is one thing that I can do to try

and put this study in perspective which would help rule out the feed-borne source for those dams, would be to look at the remainder of the cohorts which we didn't purchase -- They were left on the farm -- to see what happened to them.

In other words, if one had a case of so called maternally associated case, were those more likely to occur on farms in which those birth cohorts had also had other cases but in animals unrelated to cases. So there is that one thing.

So in terms of the maternal risk factors, as I say, the presence -- You know, that may only be there in the presence of the feed-borne source, and it may be untangleable. But it's just that all that we can say at the moment is we do not know of any kind of biological mechanisms that this thing is happening through. Theoretically, yes; but practically, no.

CHAIRMAN BROWN: Dr. Lurie had a question. Then, you know, we are getting so close to the discussion period and our speakers will all be here that I would very much like to get to it as quickly as possible, and then when we have questions that require the expertise of the people who have addressed us, we can ask them questions at that time. Peter?

DR. LURIE: This is a question for Dr.

Kahn. As you know, on December 17, 1993, the FDA wrote to a number of drug companies insisting that they no longer source their bovine derived materials from cattle which have resided in BSE countries.

I'm going to make the hypothetical assumptions that the fact that your company has made a presentation and flown in an expert from Britain that you're here for a reason that may be related to your having produced one of those vaccines. And assuming that my hypothetical assumption is correct, my question is: Why did you ignore the December 17, 1993, FDA letter?

DR. KAHN: I'm sorry, I missed the very end of your question?

DR. LURIE: Assuming the hypothetical stated, my question was why did your company ignore the December 17, 1993, letter?

DR. FREAS: Dr. Kahn, before you respond, to prevent me from reading the conflict of interest statement over again, we are not allowed as a committee to discuss individual manufacturers or individual products. We have to talk in generic terms. So her answer has to be in a generic term for all manufacturers, not for her specific company.

CHAIRMAN BROWN: Is that possible to do,

Dr. Kahn? If it's not, we'll finesse the whole thing.

DR. KAHN: I just want to say, and I'm sure this is common with other manufacturers, that we take all such letters and guidance and recommendations, as these were, seriously, and we have recommendations from multiple countries' regulatory bodies.

Our policy -- I'm sure this is common with others -- is to move away from any risk or perception of risk, and even perception of risk can be a problem today. I can say that, even as early as 1990, we made the concerted decision to make all bulk manufacturing, all routine manufacturing steps -- the serum would be sourced from countries which include New Zealand, Australia, and go away from any country that would be listed or a risk country.

Other materials would have come from Europe and other countries which were non-BSE. There's an evolution in the list of countries that are causing a problem. So we are always looking for ways to come into line.

Now having said that, for some of the source materials, assumptions were made by our company. Maybe they are considered unwarranted today, but they were made in full, good faith of disclosure

and the fact that the starting materials are non-infective. I think the non-infectivity of starting materials is the cornerstone for what you do in vaccine manufacture, and very important to us.

We have written, shared all of this information with the agency, and I can honestly -- and we also, by the way, improved traceability, decreased the chances of cross-contamination in collection. All those things were put into place as soon as any hint of a risk was mentioned from the Eighties.

SB is working very closely right now with FDA to evaluate and to implement any changes that are considered necessary to address even the perception of risk.

DR. LURIE: So you did ignore it then.

DR. KAHN: No, we don't ignore their letters.

CHAIRMAN BROWN: I think that this is a very pointed question, and what we want is blunt questions, and I think if we want to talk generically and globally, that's fine. But we can't have this dialogue with respect to SmithKline and you.

DR. LURIE; I mean, I guess the overall point, though, is that it's very difficult for this committee to make any kind of real assessment of risk.

I understand we're not voting on anything, but it's difficult even to discuss in the absence of our knowledge of what vaccines are an issue, what numbers of vaccines have been injected into people, what numbers of vaccines remain on the shelf, how long it would take for particular vaccines to replenish, you know, what the lag time would be. And all of this is beyond a theoretical discussion, and it only becomes real and our deliberations only become of much use, it seems to me, at the point that that sort of information is available.

CHAIRMAN BROWN: Well, Peter, you've been on the committee long enough to know that we never make decisions based on scientific evidence, and we're not going to start today. Thank you, Dr. Kahn.

An even shorter presentation now by Dr. Jeffrey Almond, who represents Aventis Pasteur.

DR. ALMOND: Thank you, Mr. Chairman, and it's an interesting turn that the questions have taken. I would like to say that I agree with Ray Bradley in his analysis, and one thing that I did with Ray was to work very closely with him -- could I have the first slide, please?" We have a technological failure. I think the guy from SmithKline turned my slides off.

CHAIRMAN BROWN: Can you make your points without visual aids, Jeffrey, or is that not --

DR. ALMOND: Yes. I can start making several points without the slide.

I was about to say that I worked very closely with Ray Bradley, because he, as he indicated on his slide or during his introduction, was the coordinator for the MAFF research program in the U.K. I was coordinator for the Research Councils, the Biotechnology and Biological Sciences Research Council of Great Britain, during their research campaign, and I was coordinator of that for a period of eight years and worked very closely with Ray.

I was also a member of the Spongiform Encephalopathies Advisory Committee of the U.K. and, of course, was heavily involved with all of our friends here today from the U.K. during the very heady days of 1996 and through there where we first observed new variant CJD.

I am now, however, Senior Vice President of Research and Development to Aventis Pasteur, and it seems that this subject, of course, is a broad one, and comes with me wherever I go.

I wanted to say that in Aventis Pasteur our approach to this over several years has been, as

we indicate on this slide, to check all of our processes for the production of all of our vaccines on the U.S. market at the stages of the primary or master seed lots -- and we've heard about that this morning and I've picked a few slides out that relate to where they come in, but you all know that -- the working seed lots, the primary cell banks, the working cell banks, and the industrial scale production, the purification and its effects, and of course, the final formulation. Next slide, please.

We consider every ingredient of every solution, growth medium, purification process, excipient, etcetera, at every stage of its preparation to identify materials of ruminant origin.

We then ask questions about the species of the animal concerned, the geographical origin of those animals, the date of the preparation of the material and, of course, the crucial date is the first of January 1980. Before that, we assume no risk whatsoever. After that, that is about an incubation time away from the first appearance of -- or the first diagnosis of BSE, as Gerald Wells informed us, in December 1986.

We also look, of course, at the processes used in the derivation of materials, bearing full mind

of the fact that in some cases, such as the treatment of tallow, there are very harsh processes that would destroy any infectivity.

This is not a trivial task to check all of these things, and it does involve not only checking our own records but tracing our sources and contracting suppliers of sometimes 25 years ago, and obtaining from them a original specification details of all the materials used. That has been part of our program to assess any risks that might have been present from the BSE epidemic. Next slide, please.

For any calculations that we then have carried out, we work on theoretical risks from those components, and where we have made those calculations, our assessment has been very similar to what you've heard about from Doctors Vann and Berkower.

In fact, I had a substantial experience of carrying out these risk assessments from SEAC where we looked at risk assessments of a range of things from blood and blood products through even the ash coming out of power stations where infected carcasses were being burned. So the methods that we have used are very similar to those described.

What one does in those cases is assume a U.K. origin as a worst case scenario, estimate the

relative risks to the date of the process, bearing in mind what I said earlier about the date, estimate the dilution factors where appropriate in the process, estimate inactivation of the agent by process steps in the manufacture, estimate the extent of removal or clearance of the BSE agent by purification of the vaccine active ingredients, and then assign a numerical theoretical risk to the final vaccine dose.

When one does that, we have no concerns about any of our products. It gives levels which are substantially ahead of those that we heard of before in terms of the numerical value. In other words, zero risk to all intents and purposes with very large numbers of safety errors.

So while we at Aventis Pasteur agree with most scientific experts that the risk for bovine materials in vaccines remains theoretical, we are taking steps to address concerns, and we have made progress in this direction with the goal of eliminating bovine source material where possible or by using safe sources, if removal is not technically feasible.

It has to be remembered that, for some cell culture types, there is no good alternative to calf serum. It is quite difficult to grow those cells

in the absence of calf serum, and if you try, your yields of the vaccine virus will plummet substantially, and at the present time it is not technically feasible to totally remove those bovine products.

We are committed to supplying vaccines that are safe, efficacious and in full compliance with the regulatory requirements, and we are confident, as I reiterate, that our existing products meet these standards, and there is no clinical or scientific evidence suggesting that the use of bovine source materials in vaccines presents any safety risk.

My final slide just makes the point that we believe that it is important to maintain public confidence in vaccines and in immunization, and that even a theoretical risk must be taken seriously, if it undermines public confidence.

The greatest danger that we see is the possible return of vaccine-preventable diseases caused by doubts about the safety of vaccines. That's why we are anxious to do the risk assessment and make sure that our vaccines are safe.

We are, of course, committed to working with all public health community organizations to maintain confidence in the safety of vaccination.

Thank you.

CHAIRMAN BROWN: Thank you, too, Dr. Almond. Are there any representatives from other vaccine manufacturing firms in the audience who wish at this time to make a statement? If not, we now have an open public hearing, and we are aware of two individuals who have notified us that they wish to speak.

the first is Mr. John M. Clymer who is Director of External Affairs at the Albert B. Sabin Vaccine Institute. Mr. Clymer.

Well, barring Mr. Clymer's presence, we will go on and see if the second representative is here. That's Ms. Lynn Tylczak, I think it might be pronounced, who was going to speak on the importance of vaccination. Yes, she is here. How do you pronounce your name?

MS. TYLCZAK: It's Tylczak, rhymes with "smile back." T as in Tiger, y is in yak, l as in llama, c as in camel, z as in zebra, a as in aardvark, k as in kangeroo.

CHAIRMAN BROWN: Obviously, you've had a great many questions to that effect. Please go ahead.

MS. TYLCZAK: Thank you. Good afternoon, members of the committee. I apologize. My knees are

shaking. The last time I spoke in front of this many people, I got married. So cut me a little skack.

My name is Lynn Tylczak, and I'm the Communications Director for PKIDs, Parents of Kids with Infectious Diseases. We are a national nonprofit with two missions. First, we assist the families of children affected by infectious diseases. Second, we educate the public about infectious diseases and various methods of prevention, including immunizations.

I am here to speak on behalf of those families whose children suffer from vaccine-preventable diseases. In the past few years, we have been contacted by folks from all over the country who want straight talk about childhood immunizations. These moms and dads have heard contradictory statements in the media and on the Internet, and they don't know what to believe. They want to know the truth.

As your committee ponders the issues before it today and what, if any, action should be taken to address them, we only ask that you continue to do what you have done in the past, follow the science to find the answers.

As parents, we support childhood

immunizations. It is critical for the protection of our children to maintain high rates of coverage. Too many of our families have children living with horrible diseases, diseases that could have been prevented with a simple shot.

Some of our parents even know the pain of losing a child to one of these preventable diseases. They agonize over the "if only." If only they had gotten their child vaccinated.

We all want what's best for the kids. Vaccines should not be brought onto the market until research shows that they are safe and effective. After vaccines are in use, the scientific community should continue to look for ways to improve their safety and increase their efficacy.

That said, we ask that care be taken to avoid creating fear and misunderstanding among concerned parents. Vaccines save lives. There is a big difference between inference and information, certainty and circumstances, coincidences and causal links. Let science do its job. Let it save our children. Thank you.

CHAIRMAN BROWN: Thank you very much, Ms. Tylczak. Is there anyone else in the audience who would like at this time to make a public statement on

the topic before the committee? That being so, the open public hearing aspect has been concluded, and in spite of the fact that we started an hour late, we are now 20 minutes ahead of time.

Now the fun begins. As I said at the beginning -- Let's see. Before I do this, I've been asked if Dr. Schoenberger from the CDC would get up and at least -- and not "at least," but put on the record a statement about the presence or absence of cases of new variant CJD in this country, the United States. Larry?

DR. SCHOENBERGER: Yes. We've been conducting surveillance of CJD in the country, at least since 1979 and, of course, have paid even greater than usual attention since the report of the emergence of new variant CJD in 1996 from the U.K.

I can tell you that we have not had any documented cases of new variant CJD in the United States. We are fortunate in looking for new variant CJD versus regular CJD in that there are some major differences in the age group that is affected, and that has made our job a bit easier in that respect.

As many of you may know from some of the previous slides, new variant CJD affects a much younger age group than normal CJD. The mean age for

regular CJD is in the order of 68, whereas the mean age for the new variant CJD is more like 27-28 years.

In fact, we've been dropping with time with teenagers and so on. We haven't had a single case in the United States of CJD in teenagers. We've got very good evidence on all the very young cases, which constitute perhaps 60 percent or so of the cases in the United Kingdom, and all of them have been ruled out with either tissues or very specific types of investigation.

We also have an ongoing -- There is no case in the U.K. of a case over 55 years of age at death, and we have an active surveillance now of looking at all cases under 55 for clinical and pathologic evidence.

So we are pretty confident that we do not have new variant CJD in the United States, and I gather that's what they wanted to get on the record.

There was a question earlier about what other factors in that cohort study of calves that might be different between those that have a dam that's infected with BSE versus those who have a dam that's not. In these diseases, I believe genetics do play a role.

One possibility is that there is some

increased susceptibility that is evident in the calf whose mother had BSE.

CHAIRMAN BROWN: Thank you, Dr. Schoenberger.

We have three questions that were phrased. They look a little complicated in the way that they were put together. In fact, they are not, but that will require a slight reorganization of the questions.

Before I try to reorganize those for you, I'd like to give you just very briefly my read on this issue as a way of trying to orient and focus the discussion which, in this particular case, is very vulnerable to being dilatory.

I think the first fact is that we are looking at levels of infectivity which, if present, are very, very low, and the consequence of that is we are looking at risk that is very, very low.

I think the committee would share with me the idea that the only thing worse than the death of a child is the death of a child that could be prevented. Having said that, and having listened to the lady who spoke, that is a two-edged knife. One can die from BSE or one can die from a vaccine-preventable disease.

So the discussion will invariably involve

a trade-off, as it always does, between what is at present a theoretical risk versus what would certainly be a real risk. In risk assessment in this particular instance, I don't think there probably are four. There are probably three elements, and you've heard about them all.

One is the source. One is the tissue, and one is the processing. We probably haven't heard too much about processing, because that tends to be proprietary. But let me give you an example of the kind of thinking that might go into the source and the tissue.

The worst thing would be, for example, that material were taken from a sick animal in the United Kingdom. An alternative would be -- and that would be a Category I. Another would be a Category II or III country where you had a misdiagnosis of an animal that actually had the disease or you took material from an animal that was perfectly healthy but was incubating the disease.

None of the estimates of risk that you've heard today can be precisely quantified, and I don't think the committee should get too exercised and to put too fine a point on a number here or a number there. The fact is that every risk analysis that

you've heard or you will ever hear has serious lacunae.

So we will not be able to put a number on any risk estimate under any circumstances this afternoon.

In terms of the tissue, we all know that brain is the worst, and nothing, to the best of my knowledge, originates in brain that goes into a vaccine. With respect to serum, fetal calf serum or any other kind of serum, or other tissues, you have heard that the evidence presented to date indicates that there is no detectable infectivity.

That also is a two-edged sword, and the functional word is detectable. Certainly, any level of infectivity in cattle is very, very, very low. But arguing from a rodent model, for example, in which serum had very low levels of infectivity, it required 30, 40, 60 and 100 animals assayed to detect the infectivity.

So the fact that you've got four or five cows used as an assay for a tissue such as fetal calf serum cannot -- you cannot conclude from that that the serum from any cow with this disease is not infectious. All you can conclude is that, if there is infectivity, it is at an extremely low level, which is

no surprise.

The third point is processing, about which we haven't heard too much. We know from studies of TSE over the years that these agents are phenomenally resistant to most conventional means of inactivation. For example, usual heat and formaldehyde treatment is totally ineffective in inactivating these agents.

There is, however, a process step which is used in many biologicals which removes, rather than inactivates, infectivity. That is chromatography or filtration. Depth filtration and chromatography of any stripe, we know, removes up to three logs of infectivity.

So if those steps are present in the processing procedure, they would be a further safeguard.

With those comments, I would also mention one other thing, that dilution is totally irrelevant. One infectious unit is one infectious unit, and will infect one person by definition, and it doesn't matter whether it's in vial A or vial 1,000-A. It's still there. You cannot, so far as we know, dilute out infectivity, not in this disease.

The questions that we've been asked to address have been divided into considerations of

licensed vaccines and investigational vaccines. I think, for the first part of the discussion, we should just ignore that, because the only reason for making that distinction is because the FDA would have different options in terms of what they might do, depending on what kinds of advice they get, and they have given an example of that.

For example, in a licensed vaccine they could take regulatory action. They could do product recall, package inserts, "Dear Doctor" letters. Whereas, with investigational vaccines the options would include things like stopping a clinical trial. It would also include regulatory action or modification of the informed consent.

So, really, it doesn't matter if you get sick and you are under investigation or if you get sick and you've been vaccinated, from the point of estimating risk. It really is divided only because of the options that the FDA would have that would be appropriate.

CO-CHAIRMAN GREENBERG: Can I just intervene for a second? The risks are the same. The benefits vary. So presumably, in a licensed vaccine there's an established benefit, and in an investigational vaccine there's something that is not

established. That's why it's being investigated.

I think that's why the FDA separated them, at least for the VRBPAC committee. We are very used to thinking of licensed versus investigational. So that might be a difference.

We heard this wonderful story of polio. It would be a great concern to stop polio vaccination at the moment. An investigational vaccine for there is no efficacy shown, I would be much more free to stop giving.

CHAIRMAN BROWN: Right. Well, you've blown my train of thought out of the water. I don't think, actually, we are disagreeing. That's another reason why they are different, but in terms of risk considerations, it's the same topic.

So we are now going to talk about risk considerations, and the FDA has organized risk considerations particularly along the following lines, and you've heard them repeated several times in the course of the day.

They are interested in our consideration of master and working seeds, of master and working cell banks, and of the use of calf serum and, particularly, fetal calf serum. They have actually organized it according to the chronology of making

vaccines. These would be the earliest steps; second, the process of fermentation; third, the process of formulation.

Those same considerations apply both to licensed and investigational vaccines. So I am now going to open the discussion, and if it wanders too far from the point, I'll try and direct it a little bit, but I'm hoping I won't have to do that.

Who would like to initiate the discussion? Yes?

DR. KIM: Before I answer these questions, I'd like to ask one question. That is: We heard about the infection models using cows and mice, and is there any data available whether the age of the animal make difference? For example, younger animals would be more susceptible to this disease following inoculation?

CHAIRMAN BROWN: I should add that we don't -- We can ask questions. We can attract people. We can make comments. We can do almost anything we want, and we have all of the presenters who are still here. If a question is asked by a member of the committee, whether or not it's directed to a specific presenter, if the presenter has information that would bear on it, I would hope they would raise their hand

and let me know.

To the best of my knowledge, the age of an animal is not a factor in susceptibility, but I could be corrected.

DR. WILESMITH: I think we do have some evidence of an age dependent susceptibility, but not absolute in that it does appear that calves as a group are more susceptible than adults.

We do have difficulty of looking at that in the field, because there is a break in the majority of cattle's feeding patterns. So during their sort of 12-month to almost two and a half years, they are hardly fed any concentrate. So we don't get a good look at it. But the drill in the studies that we have performed at CBO, now VLA, have involved the exposure of calves at four months of age, which is the time at which we think naturally infection takes place.

CHAIRMAN BROWN: Is that too much different, talking to the pediatricians in the group now, from virtually every infectious -- I mean, infectious diseases typically seem to -- Well, younger people seem -- No, not at all? Sorry, forget it. It's true in animal experiments, but --

DR. WELLS: Just to add a point to John's, simply that the inoculation of cattle in the cattle

bioassays at around to four to six months is largely an operational problem, in that we have to source them, get them in, and overcome any respiratory disease when they are mixed and so on before we can put them onto experiment with a reasonable assurance that they are going to survive through to the long term.

CHAIRMAN BROWN: So, finally, the answer, Dr. Kim, I think, to your question is there is marginal evidence that younger calves are more susceptible to BSE. Please?

DR. SNIDER: On that point, I think, as many of us know, clearly, there are infectious diseases that are more common in infants and young children than they are in older children. In some cases, that has to do with the fact that -- not necessarily -- the children are more susceptible than older children. But the agent is so common in population that, when young children encounter it for the first time, they just have a higher incidence.

In other cases, since humans are one of the species that are not born with the most mature immune systems, they are susceptible, more susceptible to certain infectious diseases. But again, how that would translate for TSEs, I don't think -- I certainly

don't have a clue.

With regard to answer the first question, I wonder, Paul, if you or others could make some statement about PrP and how that protein behaves or how you might expect it to behave during some of the processes that are going on early in the manufacturing process. Do we know -- You indicated something about chromatography. Could you sort of elaborate more on some of the physical/chemical characteristics of --

CHAIRMAN BROWN: I'll say a couple of things, and then I'll let Dave Bolton complement what I say, just in terms of removal.

This is -- The word sticky is usually used. It's an aggregated protein which tends to adhere to matrices, and that is why it is taken out when material is run through a matrix, whether it be a depth column filter or a chromatography column.

It can be removed as well by very high speed ultra-centrifugation, if that happens to be one of the steps -- partly removed, not totally removed. There is a strong but not universal consensus that PrP is, in fact, the infectious agent, not just a part of it. It's one or the other.

So PrP and infectivity from the point of view of tracking can be considered one and the same

thing. So what PrP does, there goes also the infectivity under almost all circumstances. Therefore, whether you detect PrP or detect infectivity, by and large, one indicates the other, and you can either try and measure PrP or bioassay for infectivity.

As I've indicated, and I'm sure Dave will agree, current tests which are an improvement, vast improvement, over tests that were in use several years ago, including the Western blot, have now instead of reaching a point where you need 10,000 molecules of PrP to make one infectious unit, you can now detect, oh, somewhere between -- You can detect infectivity at a level of about 100 to 1,000 the dilution of PrP.

So PrP is still a much less sensitive detection method than a bioassay, but it is getting better and better. Dave?

DR. SNIDER: What about adherence to cell membrane --

CHAIRMAN BROWN: I'm sorry?

DR. SNIDER: -- solubility in water versus lipid solubility?

CHAIRMAN BROWN: I could get into it, but Dave, you're here. Why don't you do that?

DR. BOLTON: Paul covered a lot of that.

one thing about PrP is it really depends on what strain of the agent you're looking at.

A lot of the biophysical characterization of PrP prions have been done with the 263K strain from hamsters, and that behaves very differently, say, than some of the other mouse strains or even other hamster strains like the TME agents adapted in the hamster.

So -- I haven't done work directly with the BSE agent. So it's difficult to say how that would behave. I'm not sure if anybody here has expertise on handling that. But in general, PrP is very hydrophobic. It does tend to cling to things, particularly it will be taken up by cells and may remain in cells for some period of time, although the replication rate is quite slow.

So although you may have the original agent sticking to cells and remaining with the cells and not being degraded, the likelihood that it will double in amount even over a short period of time is very small.

CHAIRMAN BROWN: Just for those on the committee, the Vaccines Committee, who were not aware of it, Dr. Bolton is one of the people who discovered PrP and has been a pioneer in its characterization ever since. That's why I defer to him.

DR. CLIVER: Another -- We were talking about doable experiments. We have fairly circumscribed host systems for our virus vaccines. I heard the varicella mentioned for the inactivated polio vaccine. I don't know whether FRHK-4 is used for the hepatitis A vaccine or not.

When we talk about introducing prions hypothetically with calf serum, even though I personally am satisfied that isn't going to be in calf serum, we are not really going to replicate those prions. We are only going to have a replicating system, if indeed either of those monkey kidney cell lines is producing prions as a matter of course; because the viruses, particularly the polio virus, will shut down DNA-dependent RNA synthesis.

So cell-specific proteins probably aren't going to expressed for long after polio virus cuts in as an infectious agent. So having said all that, if the prions aren't being expressed by the host cells in a condition that would allow them to be refolded under the influence of the introduced hypothetically prions from the calf serum, why there is no way for multiplication to take place.

This strikes me as something that could be easily enough determined, not at this sitting, mind

you, but not a great problem experimentally, compared to some of the other undoables that we are confronting here.

CHAIRMAN BROWN: Dave, is that -- I mean, I could envision the consequence of this. Well, just looking, you couldn't do it in cell culture, because you don't have an assay that's sensitive enough.

DR. BOLTON: Not at this time

CHAIRMAN BROWN: No. You might be able to do it in vivo. That is to say, you could inoculate a strain of BSE, say, into a variety of different species which are the source of a variety of cell lines. You could then possibly -- possibly -- determine whether or not those organs by bioassay were infected.

If you didn't have any -- If, for example, you inoculated BSE into a green monkey and then, when the green monkey got sick, you would take his kidney, because green monkey kidney cells might be used in tissue culture, and then you would assay the kidney to see whether or not there was any infectivity; and if there were or if there weren't, it would give you maybe a clue as to whether or not it would work. But to try and do molecular biology to see whether or not the protein folded in the normal cell -- how are you

going to do it, if it's not infected?

DR. CLIVER: The recipe for vaccines does not start with catch a monkey.

CHAIRMAN BROWN: All right. I used green monkey as an example. Use any species and cell that you want.

DR. CLIVER: There are cell lines that have been established for over 20 years. You can produce any given quantity of that cell line.

CHAIRMAN BROWN: Okay.

DR. CLIVER: My point is that they -- First of all, they've got to have a gene for prion production, which should be determined.

CHAIRMAN BROWN: They have to have -- I'm sorry, what?

DR. CLIVER: They have to have a gene -- In order to produce prions --

CHAIRMAN BROWN: Well, that's 100 percent. So that's a done deed.

DR. CLIVER: Well, but not necessarily.

DR. BOLTON: But they don't necessarily express it. I think that's one of the problems.

DR. CLIVER: One of the questions is, is that gene expressed.

CHAIRMAN BROWN: Well, I turn it over to

Dave. Can you design an experiment, Dave, that will satisfy our member?

DR. BOLTON: We shouldn't make this too complicated. If you take, basically, the fastest model of prion replication, in vivo in the best possible conditions, you have an incubation time of somewhere around 60 to 65 days.

The doubling rate, if you calculate that out, is somewhere between five to seven days. So when you look at that versus a cell doubling rate in culture, you can see why the past history of cell culture in prion diseases has destroyed many careers, I think. It just doesn't work out very well in cells that are expressing PrP at a normal level.

I think that, you know, perhaps for those of us who work on prion disease, it would be much better if we could get a cell line that would efficiently replicate prions, but it isn't happening now, and I think that -- So it's unlikely that that would be a great concern of contaminating the cell line with prions and having the prions be a major problem down the line in production of the working stocks and the final vaccine.

CHAIRMAN BROWN: If I understood Dr. Cliver's proposal, the first element of it was to see

whether or not a given cell had the machinery to convert a prion. Is that doable?

DR. BOLTON: Sure. Those are absolutely doable. If you look at -- Well, we looked at a few cell lines. There are a lot of cell lines that are normally used in propagating viruses just don't make very much PrP. So even if you try to pull it out of the cells by PCR, they are in very, very small amounts.

Each cell line that's used could be checked by PCR, for example, to see how much PrP it's making, and of course, the next step would be to check to see -- Let's see, you've got the vero cell, which is monkey cell -- can you, in fact, convert that PrP to a PrP scrapie.

Again in this case, you would have to look at PrP rez, the protease-resistant form, unless you want to do bioassays back into green monkeys to find out if it could convert -- say, inoculating with BSE agent into the cell line, would you get conversion?

Those are certainly doable experiments. They would be quite expensive. And it would for what?

DR. CLIVER: I'm just saying we need to get away from the conception that somehow or other prions are going to propagate in the cell culture in