changes in the normal endogenous prion proteins, and this happens in the Peyer's patches, in the spleen and in the dendritic cells and in the B cells.

Now you can also get peripheral replication in peripheral nerves. We know from the days that we used to think about this as slow viruses that neurosurgeons and neuropathologists would sometimes get infected by a percutaneous inoculation with infected tissue, and peripheral replication can occur in peripheral nerves and go centrally to the CNS. But the final common pathway of these different replication methods is that you get aggregation in the CNS of abnormal prion proteins and the phenotypic changes associated with the disease.

Can PrP^{sc} be inactivated? That's the hard part, and that's one of the reasons that we need to pay so much attention to prion proteins and to the TSEs, because classic microbicidal methods are completely ineffective. Irradiation is ineffective. Heat inactivation can be accomplished but is incomplete. In other words, you can heat this and relatively inactivate them, but you get incomplete inactivation, and chemical inactivation is highly variable.

CBER's role in the control of this problem

touches on several different products. Clearly, we are concerned about the blood supply, therapeutics, the vaccines, and now allergenics.

This is a highly selective timeline about TSE and the FDA in the United States. The first time that a letter was sent to the manufacturers from CBER was in May 1991. This alerted the manufacturers that there was a potential problem with bovine products.

In December 1993, the FDA sent a letter to all the manufacturers. In December 1997 the U.S. Department of Agriculture expanded its list of banned countries to include all of Europe. In April 2000 CBER sent another letter to the manufacturers, and as a follow-up to that letter, we in our Division sent a memo in May 2000 to the allergen manufacturers.

We also sent another memo as a follow-up in August 2000, and in the interim in July 2000 the TSE Advisory Committee and the Vaccine Related Biological Products Advisory Committee had a meeting in July that also touched on several of the issues that we are going to talk about today.

The rest of my talk at this point about TSE is going to cover those memos that we sent to the allergen manufacturers and the data that came back and our analysis of those data.

Well, how do you do a risk assessment on a biological product for the possibility of transmission of TSE? Well, you have to quantify or estimate the risk based on the animal source. Where did the animal come from, and when was that animal coming from that place?

Remember that slide that I showed sometime back that the peak periods are confined both geographically and temporally. So we need information about time and place to quantify the risk.

We also need to know about what the particular tissue is. I'll talk about this in a few minutes, but certain tissues are higher risk than others. We need to know what processing or dilution methods were gone through and how they might have impacted the infectivity.

Specifically, as we get to immunotherapy, we need to know the protein doses associated with immunotherapy prions or proteins, and they co-purify with proteins. So we need to have some estimate of what the total protein doses are.

Finally, we need to know whether are any route specific risks, whether subcutaneous inoculation has a greater or lesser risk than other forms of inoculation.

Well, let's talk a bit about the infectivity of various tissues. The European scheme is presented here with some adaptations. The scheme involves going from category I tissues to category IV tissues, where category I tissues have the highest infectivity, and category IV tissues have no detected infectivity.

The highest tissues, obviously, are brain, spinal cord and eye. Those will have the largest amount of abnormal prion proteins in them. Tissues in category II are called medium infectivity. They include a variety of other largely lymphoid tissue that has a lesser degree of infectivity.

Now the ratings of these two classifications are actually based on infectivity studies either from cattle to other cattle or from cattle to mice.

In category III and IV, there's really no detected infectivity for the most part in those particular studies, but category III tissues, for the most part, ended up in this grouping because there were good data from scrapie studies that had been done in the past that these tissues were, in fact, infective, although at a relatively lower level.

So even though the more recent infectivity

studies with the bovine TSEs suggested that these tissues were in this group, the scrapie studies suggest that they were moderately infective and should be in a somewhat higher group.

Again just one final comment about even the group IV is that the sensitivity of these kinds of studies is not infinitely low. So that's why we use the term no detected infectivity, because there are limits to how sensitive the techniques are. Yes?

CHAIRMAN OWNBY: Jay, in that category IV, is all blood and blood products contained in that blood clot you list there?

DR. SLATER: Yes, it is, but again I don't think we can be fully reassured necessarily by that. There was a recent study from Lancet that clearly showed that sheep could be infected through a blood transfusion. So there is some infectivity.

Remember, when most of these studies are done by intracerebral injection into calves or mice, you can inject up to 50 microliters of material into a mouse's brain without killing the mouse immediately. So that certainly limits your ability to detect the infectivity. Even into calves, there's a limit to the volume that you can inject; whereas, with a blood transfusion, you will obviously have a much greater

volume that you can inject.

So we have to approach all of this with a proper sense of reserve or perhaps even skepticism.

DR. SOTO-AGUILAR: Would cornea be included in the eye?

DR. SLATER: I'm sorry?

DR. SOTO-AGUILAR: The cornea?

DR. SLATER: The cornea, I believe, is included in the eye. Yes. It's the way that CJD is transmitted iatrogenically is by corneal dura mater transplants.

Now some special categories of interest. Glycerol is, obviously, of major interest for people that are interested in allergens. Well, glycerol can be obtained from both plant and animal sources, and in fact, for the most part, when we ask our allergen manufacturers, we found that they were derived from plant sources, not animal sources. So that was reassuring right from the beginning.

It turns out that, even when it's of animal origin, because glycerol is a distilled product, that the studies and the Advisory Committees have concluded that, even if glycerol is of animal origin, it's not considered to be infectious.

Likewise, milk, by virtue of studies and

other data, is not considered to be infectious. Gelatin is an additive for some of our products that I'll talk about in a few minutes.

It was originally not considered to be infectious in the first deliberations. However, as a result of some spiking studies that were done in which prions that were spiked into gelatin before its processing and taken through the entire process actually made it out through the end, there was some concern about the use of gelatin, especially in parenteral use, and in a 1997 guidance document CBER recommends against parenteral use of gelatin unless it is from certified origin.

MS. LIBERA: Can the distilling process itself remove TSE from the glycerol?

DR. SLATER: I think that's the thinking, is that the distilling process separates the prions from the glycerol.

MS. LIBERA: That's why it's done?

DR. SLATER: I don't think that's why it's done. I think it's done in order to purify the glycerol from the tallow.

MS. LIBERA: So it can be removed? So it can be in there, but it is removed in the distilling process?

DR. SLATER: Right. Right.

MS. LIBERA: A hundred percent?

DR. EGAN: Bill Egan. To answer your question a little bit with regard to the glycerol, I think it's first treated with sodium hydroxide to liberate the glycerol, and then the distillation itself, the glycerol being more volatile, would come over any proteinaceous material that stay, but also the temperature that is involved in the distillation would again inactivate protein.

So I think there are sort of three processes that are involved that would help to deactivate or eliminate any prions. So I think that's why there was -- the tallow derivatives were not considered to be problematic.

DR. SLATER: Thank you.

In May 2000 we sent our first memo to the allergen manufacturers. We asked for four different types of information in that memo. We asked for the specific animal sources that might have been used in any of the allergenic products.

We specifically asked if any neural tissue was used in any of the products. We asked for the origin and the residence of the cattle, and we asked for the dates that the cattle were obtained.

Why did we ask for each of these? Well, we asked for the country, because the risk is geographic. The risk is significantly larger from U.K. cattle, greater than EU cattle and much, much greater than from anywhere else.

We asked the dates, because the risk of TSE, especially prior to 1980, appears to be negligible. We asked for the specific tissue, because there is a risk associated with specific organs.

What did we learn? Well, for the most part -- Yes, Dr. Claman?

DR. CLAMAN: It may be a minor point. Are we sure that the geographic distribution is not the result of ascertainment bias? The countries that presumably have little or none of it, have enough samples been tested or is it based on -- Is it based on clinical evidence of diseased animals?

DR. SLATER: No. There are surveillance programs in place to collect pathological tissue. Now if you are asking what the sensitivity of the surveillance programs is in each country, I can't tell you that, but there definitely are surveillance programs in place. Is that correct? No, it's not.

DR. MIDTHUNE: Karen Midthune. I think in instances where the U.S. Department of Agriculture

felt that the surveillance practices were such that they might not be sensitive enough, those countries were also put on the USDA list as countries where, although perhaps BSE might not have been identified, that there was a risk for BSE.

DR. SLATER: Thank you. So the results of our first screen were somewhat reassuring. We were somewhat concerned that our manufacturers had specific beef extracts, but we were reassured to learn that they all obtained them domestic sources, in some cases the local supermarket.

There are some deer and deer hair and pelt and venison extracts out there. These are obtained from domestic kills, and in the particular manufacturers' case, it was from greater than 20 years ago.

Our area of concern focused on mold extracts. Several of our manufacturers use media supplements that were of bovine origin. So as a follow-up we asked for more information in our august 2000 memo, asking for lot-specific mold origin and culture information, asking for the number of lots possibly affected.

For suspect lots, we asked for certification that the material was obtained from

approved countries. We asked for quantitative flow charts so that we could calculate the risk for each product, and we ask the manufacturers to actually do their own risk assessments.

Why did we ask for all of this? Well, the lot-specific mold origin and culture information -- we really wanted to start excluding lots from consideration. What we got in the initial screen was a large number of lots in which bovine materials are used, and what we were looking for was to sort of narrow down this field of concern to products that were in the wrong geography, in the wrong time, as opposed to in the right geography, in the right time.

So we were trying to exclude lots from consideration based on known dates and culture conditions.

Why did we ask for certification? Clearly, to ascertain that bovine materials were sourced from approved countries. We asked for quantitative flow charts to help us in assessing lot-specific risks, and we were asking them to do their risk assessments so that we could both do the risk assessments and at least compare them and see where we went.

So this again is our selective timeline,

and this is where we were when we got these data. We did eventually get the data from the manufacturers, and what we are going to discuss now is how we analyzed those data and what our analysis showed.

Well, what we were looking for first is to estimate the infectivity of the media itself. So there are two stages in this process. One is to go from the cow to the media supplement, and then the next stage is going to be from the media supplement to the actual final mold product and what the risk is associated with that.

So let's take this one step at a time. The first thing we are interested in is the tissue LD_{50}s per gram. That depends on what the specific organ is of the tissue. Now, remember, we talked about category I, II, III and IV. There actually are estimated LD_{50}s associated with this based on transmission experiments that have been done.

For category I, which is central nervous system tissue, the estimated LD_{50}s is 10^7 LD_{50}s per gram. If you go down to category II, it's something under two and a half times 10^4. If you go to categories III and IV, it's between .1 and 100 LD_{50}s per gram.

The next part of the process is to

determine how much stuff goes into the lot,and we could do that in two ways. We could determine the number of grams of tissue per cow that go into each product, and then how many cows go into each lot.

Well, it turns out those data are really available with any precision only for a product that's made with a specific organ, such as BHI, which is brain, heart, infusion media. Now for BHI, or brain, heart, infusion media, we know that the cow brain is about three-quarters of a kilogram in weight.

We know, by talking to some manufacturers, that they use about 2,000 cattle per lot or something to the order of 1.5×10^6 grams of cow tissue put into each lot.

Now for other media components that are derived from skeletal muscle and mixed tissues, the specifics are really less certain. We really don't know how many cows' skeletal muscle went into this product, and certainly for gelatin we don't have a clue. But we have used this estimate of 1.5×10^6 grams per lot as an overall estimate of the number of cow tissue that goes into each lot for the purposes of our calculations.

Next we are concerned about what the risk -- regional risk is per cow of being infected with

TSE. As I said, that risk is geographic. Now at its peak the risk of a random cow being infected in the United Kingdom -- this is at the peak of the epidemic -- was about one in 100. So one in 100 cattle had abnormal prion proteins and was infected during the peak of the epidemic.

That has come down. The estimate that is currently used for the European Union is that the risk is about 10^{-4} or one in every 10,000 cattle may be infected.

For the purposes of our calculations, since none of our manufacturers use UK specific suppliers, we have used the 10^{-4} number as our regional risk.

DR. LEHRER: Jay, when you say none of your manufacturers have used UK specific suppliers, is that the same thing as saying that none of your manufacturers have used suppliers obtaining material from the UK?

DR. SLATER: Well, I think that's a very good point. None of our manufacturers are UK based. So there is no a priori reason. You see, we'll skip a little bit down the line just so I can answer your question.

What we are dealing with here in all of

the cases that I am going to calculate out is uncertainty. We have no supplier. We have no manufacturer that has come forward to us and say we know that we used a supplement that came from the Netherlands or that came from Germany. We just don't have that information.

That wasn't really the case with the vaccines deliberations back in July. There were manufacturers in which they had specific origins that were of concern. In our case, we don't really know that. So we're making a guess based on uncertainty.

Now for all we know, the manufacturers, for all their uncertainty, may have domestic products, and for the domestic product the risk is considered to be essentially zero. So we think that using the EU estimate is a good mean estimate or actually it's a good worst case estimate, we think, of what the risk would be, what the regional risk would be. But it is a guess in the midst of uncertainty. Yes?

CHAIRMAN OWNBY: Wouldn't the worst case scenario be assume that they all came from the UK?

DR. SLATER: Well, that would be a very worst case scenario. We really have no reason to believe that that's the case. I think that, in fact, when we've done digging, we have found that some of

our manufacturers have started being uncertain, ended up having domestic supplies anyway.

CHAIRMAN OWNBY: But when the UK removed so many cattle from their herds, what happened to all those cattle? Were they just summarily all destroyed or were any of them processed?

DR. SLATER: No. They were destroyed. That's a good question, but they were destroyed.

Is there horizontal infectivity among cattle? I don't believe so. I haven't seen anything about horizontal infectivity among cattle.

DR. LEHRER: Relative to that point, Jay, when you talked about wild animals being infected, I wondered if that might be the case, because with deer, for example, wild deer, I couldn't imagine how else they could be affected. Maybe you have some thoughts on that.

DR. SLATER: We don't know. We don't know what the transmission is among -- for the chronic wasting disease. This may be a spontaneous mutation. In other words, even though all organisms that have TSEs generate infectious material, some of them do develop the disease spontaneously. At least we think that that's the case.

So we don't know what the origin and

transmission of the disease in the deer and elk is.

DR. SAXON: But it's not the exact same disease as is the bovine disease. Many species have these spongiform encephalopathies, like humans.

DR. SLATER: No, it's not a virus.

DR. SAXON: Unless the Nobel Prize Committee is wrong twice and going for a third time, it's not a virus.

DR. SLATER: The next thing that we look at is process reductions in the course of making the media itself. There almost certainly are some process reductions in the making of the media, but for the purposes of our discussion today, we are considering there to be no process reductions in making the media itself. So we take that as a factor of one.

Based on these numbers, we calculate what the LD50/lot are, and then we calculate the LD50/ml of the media supplement. Not, actually, in making that conversion from the lot to the milliliter, we actually incorporate the use of the supplement in the final growth media product. So we incorporate -- You know, if you added peptone up to a 30 percent dilution, then we would incorporate that into this number here.

So this actually gives us the LD50 of the medium that is used to either store or grow the molds.

So what did we learn about the different media supplements that we were told about? What I'm listing here is all the media supplements that our manufacturers alerted us to the fact that they had bovine components.

Well, it turns out for five of them there were no bovine components at all. For proteose peptone number 2, proteose peptone number 3, peptamin, neurospora culture agar, and malt extract broth, there were no bovine components.

Now this isn't because the manufacturers were telling us misinformation. They were given ambiguous information by their suppliers. In other words, the suppliers would sort of say, well, you know, it has this category. Well, when we dug into it, we found out that these were components that were derived from other animals, not from cattle.

For three of the products, peptone, malt extract agar, and YM agar and broth,there is a bovine component, and it was gelatin. We're going to discuss those products in just a couple of minutes.

For seven products -- one of them is a broad group of products, the saborauds media, they contain bovine milk, and those were excluded from consideration as well.

Finally, when we narrowed it down, we found four products that actually contained either bovine muscle, organ tissue, or in one case neural tissue, the brain/heart/infusion media. So what we're going to focus on for the rest of this analysis is these four media and the three media that contained bovine gelatin.

Now having identified the infectivity of the media supplement, we now go from the media supplement to our final product, and the way we are going to determine that is ultimately we are going to take it through these process reductions, indicate whether we think there's a species barrier or not, a root barrier, estimate the annual U.S. dose of the product, give the LD50 per year then that's administered to the U.S. public, and by inverting that come to the number of years that we would have to go by to come to a case.

What are the process reductions? Well, the process reductions in making the mold product: You have to look at how these molds are made, and this is a very generic slide. The manufacturers all have differences among their processing, some of them subtle, some of them not so subtle.

Basically, you go from a master seed to a

working stock to a production lot. You harvest the molds, and there are various ways of harvesting the molds. Sometimes the mold mat is just taken off the top. Sometimes it is ground up with the media. It really is highly variable, and then finally there is an extract preparation.

Now we talk about process reductions and process dilutions. But really, there are some steps that lead to concentration of protein. After all, the manufacturers are trying to isolate the mold proteins and concentrate them for administration. So some of the steps, such as precipitation, drying and lyophilization, actually lead to concentration of protein.

So the process reductions were all individual for the products, and we calculated them out based on the flow charts that we got from the manufacturers.

What about a species barrier? Well, there are no data on species barriers from any animal to humans. There are data on species barriers from cows to mice. There are data on species barriers between other animals, and the species barrier appears to be something on the order of one to 1,000. But in our ignorance about species barriers to humans, we take

the worst case assumption that there is no species barrier. We think there probably is, but since we don't know what it is, we can't factor that in.

What about route barriers? Well, that there is a fair amount of data on from both cow to cow transmission and cow to mouse transmission. Using intracerebral inoculation as the 1.0 in terms of efficiency of infection, we find that subcutaneous injection has an efficiency of infection of about one to 24,500, and we use that factor in our calculations.

Now this is a very hard calculation, and that is the annual U.S. dose, because we really don't know what the annual U.S. dose of any one of these particular products is. We have, based on manufacturer data, that there are about 30 million doses of immunotherapy given every year, and now we have absolutely no data beyond this. So the rest of this is fairly, I think, conservative hand waving.

Assuming that about ten percent of the injections that are given contain some mold product or another, assuming that about ten percent of those are from one particular manufacturer, given the volume, the part of that a volume that we think would probably contain molds, we're estimating -- Now this is not for total mold immunotherapy in the United States. This

is for a particular product, a particular product or a particular product line -- about 30,000 milliliters dose of each individual product annually to the United States.

Then based on that, we go through a calculation LD50 per year and number of years to go through for each case. When all the dust settled, there were three different scenarios that we were worried about.

The first scenario is the use of an uncertified media from Category IV tissue in mold propagation. This was one manufacturer, one small portion of its product line.

Scenario number two: Use of uncertified media containing gelatin in mold seed stocks. So the gelatin was not in mold propagation. It was in the seed stocks that they started with initially. This was three manufacturers, several products in their lines.

Finally, we have the use of an uncertified media from Category I tissue -- that's brain/heart/infusion media -- used in mold seed stocks, one manufacturer, several parts of their product line.

Let's go through the first calculation.

This is the use of uncertified media derived from Category IV tissue in mold propagation, one manufacturer, one portion of their product line. I'm going to go through this particular spreadsheet in detail. The other ones will sort of follow after this.

Category IV tissue is the tissue that has .1 LD_{50} per gram. This is the no detectable infectivity. So this is the detection limits. Again, the grams of tissue per cow, 750; the cows per lot, 2000; the regional risk we're taking as the EU risk or 10^{-4}. We assume no process reductions. We come out with these numbers for the lot LD50 and for the milliliter LD50.

Manufacturer process reductions of this particular product really only reduced the potential infectivity by .3. Again, remember, some steps dilute. Some steps concentrate the protein. So in the end, when we analyzed this manufacturer's data, we came out with a dilution factor of about .3.

Again, we assumed no species barrier. This is our assumption throughout this. We assume a root barrier of one to 25,000. Again the annual U.S. dose gives us 1.1 x 10^{-7} LD50 per year or the number of years that we would have to wait to see a case is

18,500,000 years.

A little perspective: If we were going backwards, humans were thought to arrive about a million goes ago. So this is 18 times that interval. So this is a good big number. However, there was one assumption in that calculation that is highly questionable, and that is that the assumption that the material was derived with exclusively Category IV tissue.

What if, as probably was the case with butchering practices sometime ago, there was contamination of our Category IV tissue with Category I tissue, spinal cord that made its way into this allegedly Category IV tissue?

Well, remember, Category I tissue has a very, very high LD50 of 10^7 LD50 per gram. If there were a .01 percent contamination -- and this is the amount that was used in the vaccines estimates over the summer -- that would lead to LD50 of 1,000 LD50 per gram for this so called contaminated Category IV tissue.

The numbers here are all the same except we start out with 1,000 LD50 per gram instead of .1 LD50 per gram. We still come up with a comfortably large number of 1,850 years before we would expect to

encounter a case of transmission, given these numbers. Remember as well, we assume no process reductions. We assume no species barrier.

Let's go to the next scenario. This is the one where mold seed stocks are using uncertified media containing gelatin. This was three manufacturers, and this is the worst case of those three manufacturers. So we did the calculations for all three, and we came up with larger numbers, if anything, for all three of those.

We start with the tissue LD50 per gram of 1,000 gelatin. It's really uncertain what the LD50 are, but this is a good worst case scenario for gelatin. Probably it has less. All the numbers are the same. The manufacturer process reductions in this particular case were .08, since this product started out in the seed stocks, and we come out with LD50 per year of 4×10^{04} or a 5,000 year interval.

Finally, the use of uncertified media derived from Category I tissue in mold seed stocks. In this case -- this is the BHI case -- we have 10^{7} LD50 per gram, but again because this was in seed stocks, this particular manufacturer had a lot of dilution factors that went in, and the dilution factor was .00018. Here we come out with 4.2×10^{-3} LD50 per

year or 470 years per case.

So what's our summary? This is what I showed you before. Most allergen extracts are produced without any bovine components other than glycerol. Mold extracts are stored and propagated in culture media, some of which contain bovine components of uncertain origin.

The risks associated with these contaminations are minimal, and this I didn't have on the slide before: Manufacturers have been directed to assure that henceforth all bovine components be certified to be from approved sources.

Here's the question that we would like to ask the Advisory Committee at this point.

In July 2000 the TSE/VRBPAC combined committee suggested that the master seed stocks of vaccines need not be rederived to reduce the likelihood of TSE transmission. This joint committee came to this conclusion after agreeing that the risk of TSE transmission was remote, and the risks associated with the rederivation of the master seed stocks of bacterial vaccines were substantial.

In contrast, CBER does not believe that there are any risks to product efficacy or safety associated with the rederivation of the master stocks

of mold strains used for allergenic extracts.

So our question for the committee is: Does the Committee agree with CBER that the master stocks of mold strains used for allergenic extracts should be rederived to reduce the theoretical possibility of TSE transmission?

Now, remember, of the three scenarios that I showed you, this affects two of them. The first one where the manufacturers are using a questionable media supplement in the propagation -- that is not being asked now. What we are asking is about those two scenarios, one involving the gelatin, and the other involving the BHI where there is going to have to be a change in the mold seed stocks themselves to get to products that are from certified origins. That's our question.

DR. UMETSU: What were the risks that were thought to be a problem by the TSE Advisory Committee?

DR. SAXON: The bacterial, you're talking about?

DR. UMETSU: Right. In the statement they said they didn't think it was necessary to rederive the stocks, because there are risks.

DR. SLATER: The risk is --

DR. UMETSU: Rederivation was high.

DR. SLATER: The risk associated with it is that, when you rederive a master stock for a vaccine, you essentially have a new product of uncertain immunogenicity that has to be retested, revalidated, and that was considered to be compared to the risk of TSE transmission, which was quite low, quite remote. That was not considered to be worth the risk associated with trying to essentially come up with a new vaccine.

DR. SAXON: I don't quite understand. When you say you are going to ask them to rederive the stocks to get away from this risk, why can't one just replace the uncertified with certified materials in the growth of those materials versus rederive the seed stocks?

I mean, I don't understand why you need to rederive them. You think the actual mold is going to contain within it these prions? Is that the concern? Versus just replacing the media with certified, why are you going to make them do that?

DR. SLATER: Well, there would probably have to passage it to get rid of the -- You know, even if you were just trying to take the mold and put it into -- There is good reason for manufacturers not to use Category I tissues, period. There's good reason

not to use BHI. There are alternatives to BHI that they can use for the storage of these products and not lose any potency of their stored masters.

To rederive the master would basically mean to take the master and, instead of using it the way they use it as the source for all of their working materials, to actually take the master and make a new master out of it.

DR. SAXON: They are just going to grow it up, insert it and refreeze it, you're saying?

DR. SLATER: Yes.

DR. LEHRER: So is that one of the choices we are discussing?

DR. SAXON: Yes, that's what he's saying. Why shouldn't they do that, because in the bacterial vaccines they were concerned when they did that they may actually alter the basic structure in a way where you lose immunogenicity. Here it's not such a big issue. I don't know. What do the manufacturers think?

DR. LEHRER: Did you answer Andy's question, though, about is there any evidence that prions are contained within the molds or reproduce in molds?

DR. SLATER: I don't know that it's ever

been looked at in molds.

DR. LEHRER: How do prions reproduce?

DR. SAXON: They fold other normal prions. Unless the molds have a normal prion structure, which I've never heard of, they couldn't fold the protein in a mold, and it's extremely unlikely. So they probably aren't. I just was wondering what the issue was.

You really just want to change the media they are kept in and stick them over there.

DR. LEHRER: I agree with you, absolutely. It would essentially be several transfers. Not a big deal.

DR. UMETSU: So that should be one of the choices that we should be assessing.

DR. SAXON: Is that what he's asking?

DR. SLATER: That is the question. Is there a concern on the Committee between -- of our asking the manufacturers to do that?

DR. SAXON: What does it entail? Maybe someone can tell us. I mean, my sense, it's not a lot of work. You make a couple of stabs, but are we asking -- What is involved? Is someone here going to address it or will you?

DR. CLAMAN: I am sure it's more complicated than that, but I don't know how.

DR. SAXON: Well, that's what I was trying to get. Sam, you know how to do this, don't you?

DR. LEHRER: Well, I would think it's like any microbial agent, that you would streak it out on a plate. You want to get a single colony isolate and do it, you know, several times, and that would be it. Then you would grow up your master stock again.

The only thing is that the growing of fungi has a lot more requirements than bacteria. So it's not as easy as streaking out strep or staph. Nevertheless, if it can -- Obviously, it can grow on these synthetic media. So I would think that it should be able to be done.

DR. SAXON: Did the manufacturers object? Did anyone have a problem with this?

DR. SLATER: No.

DR. SAXON: Makes them look good.

DR. CLAMAN: You may have answered this. What were the risks felt to be for rederiving the vaccines?

DR. SLATER: Perhaps somebody from the vaccine side would like to address that.

DR. MIDTHUNE: Again, as Dr. Slater has pointed out, the risk calculations are based on a lot of assumptions, but in the Advisory Committee a number

of different scenarios were presented.

For example, with one of the viral vaccine scenarios that was considered, there had been use of fetal calf serum in the working seeds. For that particular scenario, it was estimated that the risk was approximately one in 40 billion vaccines being potentially contaminated.

That's when you have the issue at the working seed level. So if you remove it at the master seed level where you have many full dilutions in between, you are at even much lower risk. Thus, the Committee then considered the issue of what would be involved in actually rederiving the master seeds.

That could potentially be extremely difficult, because as Dr. Slater was saying, that could actually alter the vaccine that you ultimately end up with at the end of the process, and this would mean that you would have to redo large scale safety and efficacy studies, because this is, clearly, if you will, a standardized product. I mean, that is to say you really have very rigid demands on performance out of that product.

So in the face of a very, very remote risk based on just having an issue with the master seed, it was the recommendation of the Advisory Committee that

that not be undertaken because of the concerns over changing the vaccine at the end and, of course, having to go through extensive retesting of virtually what would be a new product.

DR. SAXON: That is very different than what we are talking about. We are talking about an allergen vaccine that's not got efficacy studies ever done anyway, and virus has got to be cultured out and dah, dah, dah.

So this -- If the manufacturers -- You know, they could look good. It's not hard. Sounds good.

DR. CLAMAN: Besides, molds change all the time anyway, don't they?

DR. SAXON: Well, that is why they keep frozen in stocks.

MS. LIBERA: This is probably is a very basic question. How often is a stock tested for TSE along the line?

MS. BRIDGEWATER: There is no test for TSE. There is no diagnostic test at this point.

DR. SAXON: You couldn't test --

MS. BRIDGEWATER: If there was a diagnostic test, we wouldn't have testing.

DR. SAXON: You could inject it into mouse

brain, I mean, the standard TSE test. But I guess it's not been done. There's no other way --

DR. UMETSU: The tests aren't sensitive enough to pick it up for most things other than cow's brain. So injecting this allergen extract, you would probably get nothing.

CHAIRMAN OWNBY: It would depend on how many mice you want to inject. You know, a few hundred, we probably wouldn't find it.

DR. SAXON: In millions, it may not.

DR. CLAMAN: There is no in vitro test.

DR. SOTO-AGUILAR: How is the surveillance done in other countries that have not been affected so far with mad cow disease? Do they have to wait until the animals get sick?

DR. EGAN: In the United States the U.S. Department of Agriculture has a very active surveillance, and they look at cows with neurologic symptoms particularly and, you know, post mortem they examine the brain, and they look for the spongiform encephalopathy.

In other countries there are also -- besides the morphological changes in the brain, there are antibody based diagnostics, but they are not as sensitive as trying to transmit, say,to mice, and

waiting a year or so and then looking at which mice then die of the TSE.

I think in the U.S. so far they have probably done around 12,000 animals. USDA has done about 12,000 animals, and they have all been negative. Then in other countries there's varying surveillances, and in most of Europe the stringency of that surveillance has picked up in recent years. It's, again, histological examination and antibody.

DR. CLAMAN: I agree with Andy Saxon. The risk of not rederiving seems to be very small. If the manufacturers don't object, and we have been told that they don't -- I haven't heard them say so -- it would seem that the conservative thing to do is to rederive the stock.

DR. LEHRER: Absolutely. I agree as well. I think we have to consider that a lot of the figures that Jay was presenting are guesstimates anyway, and even though we are trying to weigh on the side of the worst case scenario, but I mean it's like living in Southern California knowing that the major earthquake is going to come every 200 years or something, and it's really not an issue unless you're there at the time of the major earthquake.

I think it would be the same thing here.

It's very unlikely to be a problem. Nevertheless, if there is even one case that it occurs, I think it would be terrible, and particularly since the remedy does to seem to be that much of an issue.

DR. SAXON: I'll give you another way, interesting. In spite of the facts, they say there have been a case of variant CJD in this country in California. It has been pushed away, but the person came here. They didn't catch it here.

So it's more likely we will get a case of variant CJD over here unrelated to anything here, but then the person will have been on mold shots and then someone will point the finger there, probably when they ate a hamburger in Britain, you know, ten years ago.

So if it's real easy to go, we should do it, because otherwise people will start looking for scapegoats, you know.

DR. CLAMAN: Scape-cows.

DR. SAXON: I want to pick on Washington where they pick on scapegoats, since I'm here for my last time. So I think it's a great idea to do, so they don't turn around and start looking, you know, and it was probably something they did in Britain ten years ago and wasn't their mold shots.

DR. UMETSU: I would agree also that, since the risks seem to be low, that they should be rederived. But the other thing you can certainly do is, if you have a manufacturer's label the ones that are rederived as rederived, and the ones that are not rederived, I'm sure that the ones that are rederived will be the ones that will be chosen to be used.

DR. SAXON: But they only need to rederive a few. It's not all companies. Right? It's only a few.

MS. BRIDGEWATER: Right. Let me also point out, too, we're talking about non-standardized products, obviously, with the mold. There's some degree of -- I don't know if you would call it rederivation that goes on anyway, like many times when they start a new -- or manufacturers have to get a new mold seed stock, they will get it from the ATCC, and it's not that necessarily they would get the same lot every time.

So there's some variability in the process already.

DR. LEHRER: But I thought they would rederive it from their master stock.

MS. BRIDGEWATER: Yes. My point was that there's some variability in the process as they do it

-- as it's normally done anyway. So we're not introducing something new, but yes, we want them to rederive from what they have, if possible.

CHAIRMAN OWNBY: But when they rederive -- and my understanding was a lot of people don't maintain these real long term, that they often purchase from ATCC -- the question is then do they have to seek certification from whomever they are purchasing from that this has been propagated in materials that don't contain bovine materials?

MS. BRIDGEWATER: Yes, they do have to have that certification. They get it from ATCC. Now some of the manufacturers have stocks that they have had that go back to the Seventies and Eighties that they have maintained for a long time.

CHAIRMAN OWNBY: I think there's a consensus of the committee to request the manufacturers to rederive these, unless there's some reason to consider them exceptionally onerous compared to what we've heard so far.

DR. SLATER: Let me just ask for a slight wrinkle in the discussion, if you don't mind.

What we have been using -- What I have been using as rederivation is taking the existing master and passaging it several times to get it into -

- to make a new master.

What that really does -- and this is something you discussed before -- is that adds several dilution steps. Now it's several multi-log dilution steps. So this is definitely a good step, and really would -- because with each passaging there will be some small amount of carryover, but this adds several dilution steps to the process.

That is the question that I was asking. There is another way to interpret rederivation, and that is actually a thing, you have to go back out and find new mold and find new mold isolates. I just want to make it clear that that was not what I was asking at this point.

I would like to clarify that what we are talking today for rederivation is really introducing several dilution steps to reduce the infectivity of this starting product by several logs.

DR. SAXON: It would be many logs, Jay. I mean, not several. If you are going to pick a colony, how much contamination by the time you grow it up several times is going to be huge. It's not just -- You said -- I mean, 10^6.

DR. SLATER: I'm comfortable with what you are saying. I just want it to be clear in the record

that this is what we are talking about.

DR. SLATER: It would be an enormous change.

CHAIRMAN OWNBY: And you are going to provide the manufacturers some guidance as to how many steps are going to be required in this? It seems to me, if I was a manufacturer, the first thing I would ask is how many times do I have to repropagate this before I can now say this is a certified, safe master?

DR. SLATER: Yes.

DR. SAXON: Two times will be at least a millionfold.

DR. CLAMAN: Is this a recommendation or a directive? What are you doing, talking to your lawyer?

DR. SLATER: I am talking to my best friend at the moment.

This will be a recommendation from the committee that will be considered by us, and that we will discuss and go from that recommendation from the committee.

DR. UMETSU: Are these also organisms that the ATCC carries? Is it something that, if you have a derived stock, that the ATCC can supply to the manufacturers?

DR. SLATER: What I didn't explain in great detail when I was talking about how different the different manufacturers handle this: Only some manufacturers actually maintain their masters internally. Many of the manufacturers get their masters from outside sources.

In some cases, it will be the outside sources that they will have to go to, to get them rederived.

DR. UMETSU: But can those be standardized in some way?

DR. SLATER: Well, standardized is a loaded word, especially --

DR. UMETSU: Or certified?

DR. SLATER: -- as we discussed at great length last year. The outside sources, the sources that are used, do to a greater or lesser degree certify the identity of the mold strains, if that's what you are asking.

DR. UMETSU: Well, but now can they be certified in terms of TSE?

DR. SLATER: Well, they will have to be. That will be --

DR. UMETSU: So that would help in your directive or recommendation to manufacturers?

DR. SLATER: Yes.

CHAIRMAN OWNBY: Anymore burning comments? We were directed to have 30 minutes here for public comment. Bill, have we received any requests for public comment?

DR. FREAS: I have received no responses to the Federal Register announcement, but if there's anybody here who would like to make a comment at this time, we welcome comments from the public.

MS. WILLIAMSON: Can I just ask a question? My name is Shirley Williamson, and I'm with Holister Stier Laboratories.

Listening to the conversation just to add a couple of points, we currently have 32 master stocks that we maintain at our facility. I think we have pretty much reached the conclusion that we will be rederiving and have started that process.

At our current schedule, it will take us about a year. It will take us the entire year. So from our standpoint, that's what we are talking about.

CHAIRMAN OWNBY: Thank you. Any other comments? That answered your question? Good. Any others?

Okay, I think we can move on then with our agenda. The next was the presentation on the

statistical power of clinical studies comparing allergen extracts by Dr. Lachenbruch and Jay Slater. Jay, you are going to introduce this?

DR. SLATER: Thank you. I am Jay Slater.

Dr. Lachenbruch is going to give the main body of this discussion, but what I wanted to do was I wanted to introduce his remarks, and I am actually going to come back and say something briefly after his remarks.

The source of this topic for presentation to the Advisory Committee is that it has come up a number of times in the last couple of years that we have had bilateral discussions with manufacturers about just these specific issues that we are going to introduce now, and since there has been some misunderstandings from the manufacturers, we wanted to discuss the issue of clinical bioequivalence as fully as possible in a public format for the Advisory Committee's discussion as well, so that these concepts could be discussed and aired.

I am going to give here a generic, fictional example in which this kind of question comes up. A manufacturer wishes to change its approved production method for standardized fedweed pollen extract. Fedweed is a joke, okay? I was trying to

think of some funny name for a pollen, and no matter what funny name you think of, they are all there already. So this is the only one that I could think of that hadn't been taken by nature yet.

CBER acknowledges that existing in vitro potency tests will be applicable to the new preparation. After all, it's a new preparation of an existing product. However, CBER requests that the manufacturer demonstrate compositional similarity by the parallel line bioassay.

Now what does this mean? Well, again we are going to go back to the intradermal skin testing protocol. This was the original report. It was in November 1982 in JACI from Paul Turkeltaub, Dr. Rastogi, Dr. Baer, Chris Anderson and Phil Normal from Johns Hopkins.

This is the study method in which you do serial dilution intradermal skin testing looking at the erythema, and looking at both potency and compositional similarity using this. This paper was published in 1982.

Now about a decade later, this method was incorporated into the methods of the Allergenic Products Testing Laboratory which was actually announced for release in an FR notice in November

1994. I just want to quote a small section of that protocol for you to understand where the problem now comes in.

This is part of that protocol, Quantitative Intradermal Procedures for Determining the Relative Potency and Compositional Differences of Allergenic Extracts, quote: "At least four subjects are required per assay. One or more test extracts are to be compared against a reference in each subject. For evaluating compositional differences, subjects selectively sensitive to specific allergens in the crude mix should be selected. In order to enhance the detection of compositional differences, subjects can be selected who differ widely, 10-10,000-fold, in their allergen skin test sensitivity to the test of reference extracts."

So according to the protocol, applied correctly you can adequately look for compositional differences between two products by testing four study subjects.

DR. SAXON: Jay, what does that mean, specific allergens in the crude mix?

DR. SLATER: Yes, because the --

DR. SAXON: You're going to have to know their dura-p 1 -- I mean, the actual allergens in

there, because there's multiple, ragweed, you know.

DR. SLATER: Right. So to evaluate compositional differences -- in other words, whether allergen mix contains allergens A, B, and C -- you need to look for individuals who are selectively sensitive to those in order to detect the differences among them.

It's hard. I mean this is hard, but the problem that we've been getting hung up on is the question of four study subjects.

Now since 1993 and 1994, FDA has evolved, has refined its approached toward this issue of compositional similarity. One example is in April 1996 a guidance document appeared concerning the demonstration of comparability of human biological products, including therapeutic biotechnology derived products, and more recently in 1998, an international conference on harmonization document appeared -- this is the E-9 document that is included in your pre-package -- entitled "Statistical Principles for Clinical Trials."

One particular portion of this addresses the kinds of statistical considerations that would drive this kind of analysis.

Now before I turn the podium over to Dr.

Lachenbruch, let me just make a brief statement about what this is not about. We are not talking about lot release. Lot to lot consistency within a given product is established by accepted in vitro equivalence testing.

In these examples, we are addressing the bioequivalence among different products for which there are proposed changes in source materials, manufacturing or stabilizers or diluents.

In fact, one of the things that we showed in our analysis of the currently available standardized product is that within a standardized product, there is a tremendous amount of consistency, both in composition and in potency. But here we are talking about situations in which the manufacturer has asked to change part of the process.

Once you start changing either the source materials or the manufacturing or the packaging or the stabilizers, you have to demonstrate that the new product is compositionally similar to the licensed product, and that's where this discussion is coming from.

Now I would like to introduce Dr. Tony Lachenbruch who is the Director of the Division of Biostatistics at CBER.

DR. LACHENBRUCH: Speaking as somebody who went through the 1994 January 16th earthquake in Los Angeles, I don't want to have another one like that. It's rather exciting. I don't think I've ever been quite so scared.

Okay. Well, in abstracting this to the simplest possible situation, we are really looking at -- let's say we are looking at a single response, and what we are trying to say is are these similar? Are the responses similar?

One of the most common mistakes that is made, and I have made it myself, is oh, I'm going to test a hypothesis that the means are the same, and failing to reject that, concluding that the two responses are the same. This is a problem, because failing to reject, failing to see evidence of a difference is not evidence of no difference.

So what you do is you set up your hypotheses so that, when you reject it, you are forced to the conclusion that they are the same. So the null hypothesis is going to be a little bit difference, which says the products are different by a certain amount, and therein lies a tough situation.

So let's say we think of applying an antigen to the back of a subject. The application

should be randomly determined, and this assumes that there are no systemic effects that could muddy the comparison.

So after a period of time, we look at the size of the wheals and measure them, and we need to show that the wheals are within some small limit of each other. The question is, first of all, what is small? That's a real headache. And what's the correct method? I've already hinted at that.

So we might set the margin of difference to be ten percent of the mean of the standard. so if you typically would see a three centimeter wheal, you would want it to be no more -- the differences in size of wheals to be no more than .3 apart. So we are setting this -- we can set the margin as a fraction or sometimes we could set an absolute value for the margin, which might be based on a lot of previous experience. So it may not differ very much from the fraction method.

So we are requiring the difference of the means to be within a margin of zero. That is this margin here. So, for example, here is our picture, and we want to show that we are between -.3 and +.3.

The heavy lines would indicate a difference in the mean level. So what we are saying

is the heavy lines here are basically what the null hypothesis is that we are either more than -- that the new is different from standard by at least +.3 of a centimeter or -.3. So we want to show that the true mean difference is in this lightly shaded area.

Well, let's supposed we found that the mean difference of the pairs and their standard deviation were as follows. We found the average difference. This is a sample quantity. It's .2, and we found the standard deviation of 2.5 for this difference. We had 100 pairs. so we found a t was 0.8.

Ah, okay. If we did the standard null hypothesis, no rejection, we would say there's no rejection. We'll conclude that these are the same. However, we can always choose a small sample size and find that there is -- have no power to detect a difference.

So we said in this case, let's suppose the historically known wheal size was three centimeters and ten percent would be three-tenths. So we can compute a 90 percent confidence interval, and note that if it is entirely contained in the equivalence region -- it has to lie between -.3 and +.3 -- then we can conclude safely that we do have equivalence.

So in this case we compute a confidence interval on this basis, and we see that it runs from -0.215 to +0.615. So we could not conclude that the new and old are equivalent.

So here, for example, we see it in pictures. From -.3 to +.3 is our acceptance region, and our observed confidence interval goes from -0.215 to +0.615, and they don't overlap. We can't conclude equivalence in this case.

So either another study would have to be done or the product might, in fact, need to be reformulated.

Another method that is sometimes used, and I think this is the method that is referred to in the material you received -- it was proposed originally by Don Sherman at CDER -- and it says let's test a joint null hypothesis. The first part is that the mean difference is greater than +delta. Delta in this case is the .3. Or that it's less than -delta. That's the negative .3.

If we reject both of those hypotheses, then we conclude that the mean difference is less than delta. So notice, what we are saying is the null hypothesis is the difference is bigger than delta. The alternative is that it's the absolute difference

is less.

So let's just talk briefly about the conclusions. In any situation when we deal with a sponsor, we need to discuss the selection of the margin, ten percent, an absolute figure, whatever it is. This does need to come in, and I think sponsors should also do themselves a favor. In case somehow we don't mention it, then they should ask us what's going to be okay.

The sample size is going to be based on a null hypothesis that they aren't equivalent. You can either use a confidence interval or the two one-sided tests. There are lots of headaches in doing these studies, because typically in a short-a-difference type of thing, the null hypothesis is that they are the same.

If you have imperfect experimental treatment -- imperfect execution of your study, they tend to push the means from both groups toward one another; whereas, if you do that when you are looking at the equivalence studies, bad execution pushes the study in the wrong direction. It's favoring the alternative hypothesis, and you want to be very cautions about that.

So you should use both intent to treat and

per protocol analyses and examine them, and you need to provide evidence of trial validity, both in looking at the design and in the efficacy of the control. For example, if you normally saw a three centimeter wheal and instead you ended up seeing a 10 centimeter wheal, you would be concerned that somehow things weren't behaving properly.

Is this the last one, Jay? You've got all the other slides in there, too? Okay.

In your handout, I believe, there are many other slides, but I wanted to stick with these 11 slides, because the others are basically going through a calculation. So I thank you. I'm available, and Jay is available to answer questions. But you are going to say a few words.

DR. SLATER: Thank you, Tony.

So what I wanted to do in the next few slides was just to come back to our specific example and give some specific number calculations that might give you an idea of what this means in terms of the specific proposal. So let's go back to our fictional manufacturer.

Based on the 1993 protocol, the manufacturer proposes to enlist between four and six study subjects of varying levels of sensitivity to

fedweed pollen, and the manufacturer says that if the slopes of the two preparations are not significantly different in these study subjects, the manufacturer will then conclude that the two preparations are compositionally similar.

Now as Tony has said and as I'll repeat, the failure to demonstrate a difference does not mean the demonstration of similarity, and that's an important point to continue. But just for the purposes of putting numbers in this, let's say that the mean slope is about ten, and let's say that our acceptable differences between slopes is about ten percent or one of a difference in the slope.

Again, let's say that the standard deviation of the slope is one. We have an alpha of 0.05 and a beta of 0.2. Now you will recall that Dr. Lachenbruch provided you with these formulas, or were those in the slides that you didn't show? Oh, okay. He didn't provide you with these formulas, but these formulas can help determine the significance using the two one-sided test approach.

To give you a sense of what this approach might mean in terms of the study size, what we are really interested in is n, and now this specific rearrangement is one that I think we have to put a

caveat in here, that this may not be applicable in terms of all of the analyses; but if you rearrange the numbers that we have, we can come up with a formula for n that is this one to determine the number of study subjects, n, required to detect a difference, delta, with a confidence of alpha and a power of one minus beta using the two one-sided test approach.

This is one formula for n. It's 2 x the Sigma-squared, which is the standard deviation, times the square of the sum of the two z values over delta squared. Now if you solve this for alpha of .05 and a beta of 0.2 and reduce it, what you find is n is a constant factor 12 times the square of Sigma over delta.

Now, remember, Sigma is the standard deviation of the test that you are using, and the broader it is-- Yes, Andy?

DR. SAXON: A beta of .2 is an 80 percent confidence of finding it, if it's there. Is that right?

DR. SLATER: It's 80 percent power.

DR. SAXON: Oh, right. That's the 80 percent power. Okay.

DR. SLATER: Right. But the important thing to note here is that what's important in

determining n is the ratio of Sigma to delta, of the standard deviation of your test to the acceptable differences, and in fact those as the square of those two.

So we go through some number crunching to give you an idea of what we are actually talking about here. Let's plug in some of the numbers. In the specific example that I showed before, we -- For all of these, we accept an alpha of .05 and a beta of .2. These are all fairly conservative assumptions and default positions.

Let's say that the delta is 1, and let's say that the Sigma is 1. In other words, for this test the acceptable difference is equal to the standard deviation between the two.

Well, if you plug that in, it doesn't take much arithmetic to show, if Sigma equals Delta, then n is about 13. Now let's say that the Sigma is larger than that. Let's say that the Sigma is twice what the Delta is. Well, in that case Sigma over Delta is 2. That squared is four, and we come up with an n of 50.

Well, given an acceptable delta of 1, how low would your standard deviation have to be to get to that four to six study subjects? The answer is you have to have a pretty low Sigma. You would have to

have a Sigma that was just a little bit over half of what your Delta was.

Now, really, those are all the numbers that you need to look at this, but we can actually take it a step further. Let's say that we decide that a Delta of ten percent is too restrictive, and makes very little sense. So we are going to increase the Delta to 20 percent.

Well, if the Sigma is 2, well, then you have the same situation you have up here, and you need 13 study subjects.

Well, with a Sigma of 2, how liberal would you have to be with your Delta to get down to that level of n of four to five, and again you would have to have a delta that was pretty substantial, 35 percent of what you were looking for.

DR. SAXON: In terms of erythema, it turns out it would be 2 millimeters?

DR. SLATER: No. This is a slope. Remember, when you're looking at compositional similarity of differences, it's actually a slope. So it's a ratio of the erythema over the log dose.

So in conclusion -- and again this is something I know we've repeated a couple of times, but it's important to know that the failure to demonstrate

a difference is not sufficient to demonstrate equivalence.

Given that, for the parallel line bioassay, in general, the Sigma is at least equal to but greater than Delta usually, the number of study subjects required to demonstrate equivalence will usually exceed the four to six.

The bottom line is that the four to six that was shown in the '93-94 document will only be sufficient if it leads to a sufficiently powered study, and that the subsequent documents give good guidance as to how to sufficiently power the study.

We do ask that the Advisory Committee discuss this. In particular, we request that the Committee discuss CBER's current approach to clinical bioequivalence studies as it applies to allergen extract studies.

Are there any specific questions for me or Dr. Lachenbruch?

DR. SAXON: When they did the original study with Turkeltaub and Baer and Norman, did they actually do a power analysis like this? Where did the four to six come from? The back pocket?

DR. SLATER: I wouldn't say that. They did a large number of these studies. They did a lot

of intradermal skin testing. They had a significant amount of data. I don't know the basis of the choice of those numbers.

DR. SAXON: Because today you would get your grant rejected without a power analysis. You couldn't get it through. So I mean, you're basically saying you need to do the basic power analysis to test the hypothesis. Right?

DR. SLATER: What we are saying is the study has to be sufficiently powered to demonstrate that they are not different.

DR. SAXON: You couldn't get a grant funded today without that in your grant. You can't just say I'm going to do 12 subjects. You've got to have the analysis.

DR. SLATER: Dr. Lachenbruch?

DR. LACHENBRUCH: I believe Dr. Rastogi was involved with this and would have done the power analysis. That original study, the 1982 paper, I think, was more directed toward showing a difference rather than an equivalence study, because around 1982 was the beginning of our interest in showing no difference, and then about 1987 was the Sherman paper, and there's been a very, very active interest in that in later years.

DR. SAXON: So you're saying the original study with looking at the hypothesis being one side being different could be powered with four to six.

DR. LACHENBRUCH: Possibly, yes.

DR. SAXON: But you've just shown us that it won't work with --

DR. LACHENBRUCH: In each instance, you have to go through this analysis and see where you go.

CHAIRMAN OWNBY: So I guess the real question that you would like some guidance on is the expense to manufacturers if they did bring a new process of showing equivalence with a much larger number of subjects than we have previously had versus the concern that with such a small number of subjects we might not -- we would be woefully underpowered to detect differences. Is that correct?

DR. LACHENBRUCH: I am sorry. I didn't hear the beginning of the question. I was trying to answer another question over there.

CHAIRMAN OWNBY: Well, it seems like this is trying to balance the time and expense that it takes to do a study with a large number of subjects versus the current practice which we think is woefully underpowered to detect problems or to really establish that there is bioequivalence.

DR. LACHENBRUCH: It's answering the wrong question, I think. It's saying are they different and, if you don't say they are different, you can't conclude they are the same. Whereas, if you test are they different and you reject that, you can say that they are basically the same.

One of the things that I would point out is that Dr. Slater's sample size calculations were for unpaired data, whereas often, if you are painting two sides of the same back, you can take account of the pairing, and that will usually reduce the variance or the standard deviation quite a bit. So you are then less interested in saying it's within a certain tiny amount, but you might be able to take a larger margin relative to the standard deviation.

Your study will be much more sensitive, because your standard deviation of the slopes would be much smaller.

DR. SLATER: I think the key thing from my point of view in terms of the discussion is that a manufacturer -- and manufacturers have done this. They have come to us with the '94 methods manual and said, well, here we are following it A, B, C and D.

We simply wanted to alert the committee to the changes that have happened since 1994, to the

changes in thinking that are not being, by any means, specifically applied to allergenics and, in fact, you know, are -- but we are definitely interested in applying them to allergenics now, and we wanted to air that thinking for the Committee and, if possible, get some feedback from the Committee and some discussion about the concepts that we have introduced here.

DR. UMETSU: I think that the idea of proving nonequivalence versus equivalence is a very clear one, and it sounds like it should be implemented.

Could you give me an idea of the number of patients or subjects it would take? Clearly, the old standard was four subjects. In your estimation, how many subjects would be required to prove equivalence?

DR. SLATER: It depends on the slope, and it depends on the standard deviation, and the standard deviation could easily change, depending on the population that the manufacturer chooses.

DR. UMETSU: But are we talking about 50 subjects or 100 subjects?

DR. SLATER: Yes.

DR. SAXON: Do you think that is reasonable?

DR. SLATER: I am not being evasive. Yes,

it could be 50. it could be 100.

DR. SAXON: That's the concern. It may become unreasonable. How do you get 100 subjects? I mean to do this, it seems unreasonable at that point.

DR. SLATER: Unreasonable in what sense?

DR. SAXON: Unreasonable to get 100 subjects who are -- Let me figure something that's not that common -- who are saltgrass or saltbush positive, and they are going to have to titer 100 people when they make a change. It would certainly keep me from making a change.

DR. SLATER: Well --

DR. LACHENBRUCH: Our answer may, in fact, depend on the prevalence of the condition, too.

DR. SAXON: Prevalence isn't going to let you be anymore accurate.

DR. LACHENBRUCH; No, but if you have a condition in which you have hundreds of thousands of subjects who are affected versus something that may be 1,000 in the country are affected, your answer may be different.

DR. SAXON; Well, let's take even a common one, cat, and you were going to change our cat thing. You're going to have to take 100 subjects and do these titered back responses on 100 subjects? Have you done

100 subjects? Has anyone here? I mean, Turkeltaub started this stuff, I guess. I don't know if he did, but has anyone actually taken 50 or done a big study?

DR. SLATER: We are budgeting our estimates for cockroach at about 200 study subjects per American and 200 for German.

DR. SAXON: Two hundred?

DR. SLATER: That's just based on our initial estimates.

DR. SAXON: And you're going to do it -- What are you going to do with the 200?

DR. SLATER: Well, we are going to be determining the potency and the -- the relative potency and the potency of different extracts. This is not the same as determining compositional similarity and differences, but I think in order to do an adequately powered study, you need to do the calculations of what the standard deviation is, and you need to power it adequately. Otherwise, you just can't learn the information that you are trying to learn.

DR. UMETSU: So you estimate that it will take 200 subjects?

DR. SLATER: I'm starting -- In fact, what you need to determine is what this Sigma is for the

study in the study population, and we don't really have that yet. This is just a starting estimate.

The other point is that this wouldn't come up with saltgrass. This would only come up with a standardized allergen extract at this point.

DR. SAXON: But aren't they all going to be at some point? Isn't that where we are going?

DR. SLATER: That is the direction.

DR. SAXON: It just strikes me that -- I mean, I understand the science. You know, it's great science, but then, you know, there's got to be a better way. If you're going to come up and tell me that I've got to test 100 people for cat -- and again, I'm not a manufacturer, but I can't imagine they would have as quality controlled people as you do.

I mean, they are going to be less tightly controlled internally, I suspect, than your lab, Jay. Your la should be the highest quality, and it's tough. I mean, hiring people, getting good people. I think it's going to be --

DR. CLAMAN: I think so, too. I think that with a larger --

DR. SAXON: The delta is going to be huge.

DR. CLAMAN: The larger the number of subjects, you may be building in an extra amount of

biological variability, and the variability won't be in the patient.

DR. SAXON: We should -- Do the manufacturers actually do this test or isn't there a company that they farm it out to?

CHAIRMAN OWNBY: Well, isn't that, though, a business decision for the company as to how to approach this problem. But it seems to me there's two issues we've brought up.

One is that under the -- that ideally here, the smaller number of subjects that can be achieved, the smaller your variation. Isn't that true?

DR. LACHENBRUCH: Right.

CHAIRMAN OWNBY: That the smaller the standard deviation, the fewer subjects you would have to have.

DR. LACHENBRUCH: Right.

CHAIRMAN OWNBY: So therefore, the more precisely you do the study, the more economical it becomes. So that, I think, would be a good principle that we would have.

The other thing we've talked about is biological diversity, that skin testing people from different ethnic groups might produce different

results, and you would like to have a sample large enough to incorporate some of that variation. With, obviously, four or six subjects, that becomes very difficult to do.

DR. CLAMAN: But one of the handouts we got had some very good discussion of sequential analysis. Can't you -- If you're asking whether the number of subjects ought to be increased, I'd say yes, that's a great idea. But that's open-ended. What do you mean by increase, 100, 200?

DR. LACHENBRUCH: Is this one of those that I handed out? I don't remember talking about sequential analysis.

DR. CLAMAN: Well, can't you determine as you go along how many you need by, let's say, doing five or ten and, if the standard deviation is very small, then you say to yourself that's enough?

DR. LACHENBRUCH: You can do something like this, if it's in a formal plan. You can't do it if you say, well, I'm going to start out and then I'm going to look at it this time, and then I'm going to look at it again. You can't do it on an ad hoc basis there.

DR. CLAMAN: Well, I understand that. But you could build it into your protocol.

DR. LACHENBRUCH: There are procedures.

DR. CLAMAN: Of course, there is.

DR. LACHENBRUCH: Sure. Yes.

DR. SAXON: Dennis, this is our last chance here, you, me and Henry. So I would --

CHAIRMAN OWNBY: They will have it easy after this, aren't they?

DR. SAXON: This is great. One thing is, you know, I think Den made a great point. If you could, number one, try to get the technique with better precision, because that will cut your delta down. The other is I think this technique started in 1982. I think it's time to look for another method.

I won't suggest one, but this has been going on since 1982. It's almost 20 years later. Let's find a better. We have a new Chief of Allergy here now and lab. Find a better assay than this method measuring wheal flares. I would strongly recommend that, and I see my colleague over there shaking his head yes, too.

DR. SLATER: I agree, and actually, this method was not without controversy when it was introduced. I mean, there was tremendous debate about it, and it was implemented in spite of that, and unto this day even, to be honest with you, my question

about biodiversity in this method, I think, I'm still not satisfied with the answer, and particularly in today's environment.

Even if we didn't think that it was important, I think it's extremely important politically. Be that as it may, I think it's extremely important, period, and I'm not convinced that it's being done, particularly in very dark pigment individuals. It's just very difficult to read flares, or impossible.

Many of these are the target population, particularly cockroach. I think -- I mean, we've been skirting around the issue. You know, Andy has a way of hitting it. I think he's absolutely right.

DR. SAXON: As we end this panel, so to speak, I think one of the things this Committee should do is maybe get together with the FDA and get together a group of people who are interested in this with the Academies, whatever, under the FDA to have a go at this again after 20 years.

Should there be a new method using transgenic mice, some method? It's not going to cover -- That won't cover the biodiversity, but at least it will allow you to get the standardization in vivo system. No one likes in vitro -- some in vivo system,

but give it a day or day and a half of think tank and get rid of this.

CHAIRMAN OWNBY: No other comments? I'm not sure we've given them the answer.

DR. SAXON: Dennis, remember, they do whatever they want, no matter what we say anyway. But I think the answer is, you know -- right, Jay? Jay, I think the answer that -- we all appreciate the need to power studies appropriately. Every scientist knows that today. Your point is very well made, right, that they are underpowered.

They need to make -- You got to make it more precise. Henry's got at least -- Then as you get the deltas, you go through the study, you'll know what is reasonable. But you need to find ultimately a better way at this, because you are going to find ones where it's 200, and manufacturers aren't going to be able to do 200, are they? Not really. Probably not, not accurately like you will.

DR. SLATER: Was that a question for me?

DR. SAXON: Yes. What do you think? Can you imagine saying, you know, you need to do 200 cat, and then they are going to have technicians who aren't as good as yours, who haven't really got the --

DR. SLATER: Well, remember that the 1993-

94 manual has in it a qualification step for anyone performing this assay with sequential histamine skin tests. So if one is following the '93-94 protocol, one has already narrowed the range in terms of the technical proficiency of the individuals performing the test.

I appreciate the comment about the high quality of my lab, and I can accept that without further comment. But I also accept that these are difficult studies to do.

There is, however, probably an irreducible sigma associated with these kinds of measurements in human skin, regardless of the pigment. That, I think, is where the numbers come from, and I think the numbers are driven by the imprecision in the human skin response to injected allergens and by the difficulty in measuring it with reproducible accuracy. But we do try to get around that with the proficiency testing for the technicians, which, quite frankly, some people have to repeat several times before they are demonstrated to be proficient.

It's not a trivial step. So there is sort of a training step built in that, I think, tries to get around that problem and tries to bring everyone up to a certain proficiency level.

DR. SOTO-AGUILAR: Could CBER conduct the studies or each manufacturing company would seek patients who are very allergic to their products and then go forward with their protocol?

DR. SLATER: I'm sorry. You are asking whether this is centralized?

DR. SOTO-AGUILAR: You are trying to find bioequivalent potency for standardization of extract. Would CBER conduct the study, given products from all these manufacturing companies to multi-center to conduct the studies with two or three samples from three different companies and your own standard, or each company is going to be invited to do it on their own and then send the results to CBER?

DR. SLATER: The model is for CBER to coordinate the studies, and the data and the data analysis would be done by CBER. Within our current constraints CBER itself, meaning me, could only do testing in Bethesda, Maryland, in an area that would attract individuals from around that area.

It's important that we have both a geographically and ethnically diverse population. So at a minimum we would need to contract to other sites around the country.

I assume you are asking now about

cockroach. Is that correct?

CHAIRMAN OWNBY: No. I think we've got at least two different questions here that are not very clear. The question is, if a manufacturer wishes to change, for example, their source materials, then this is entirely incumbent upon the manufacturer, isn't it, to do the testing; whereas, with standardization of cockroach, you are talking about coordinating it through CBER.

DR. SLATER: Right. These are two separate issues. These are unrelated issues.

CHAIRMAN OWNBY: It seems to me that there is -- One of the things we haven't asked, we are just presupposing that this is the only way to get a bioequivalence, and Andy was saying, you know, can't we come up with a different system? Is it worth discussing that or is that just not an issue currently?

DR. CLAMAN: I think it would be difficult between now and lunch to define the new system that we think ought to be put into place.

DR. SAXON: That is why I suggested that the FDA -- someone like Jay pull together a -- You know, it's an opportunity.

DR. CLAMAN: Do you think we need a better

system?

DR. SLATER: Cockroach will be my first firsthand experience with the existing system. I think, as with any system, I think there are strengths and weaknesses associated with it.

Certainly, it is a cumbersome system, and certainly, our discussion today suggests --

DR. CLAMAN: And it may get more cumbersome.

DR. SLATER: -- a more cumbersome than we originally thought. That being said, the strength of the system is that it is a clinical measure of overall allergenicity and, if done properly, it can give you a good mean assessment of what the consequences are of injecting this material into a population -- into an individual who is highly sensitive.

Remember that the default position is to pick highly sensitive individuals for study in terms of standardization. Now in terms of assessing compositional similarity and differences, you are supposed to take a range of sensitivities.

I must say, it does have a fairly compelling strength, and that is that it does seem to give us what the potency of a product is in an allergic individual and in an allergic population.

But I'm very open to further discussion, both with the members of the Advisory Committee and with others, about alternative approaches that can be used.

There are weaknesses, as you all know, to the purely in vitro approaches that really preclude adopting them as our gold standard for allergenicity, and I don't think anything Dr. Saxon said was aimed at pushing that idea. Is that correct?

DR. SAXON: No, but I can give you one idea right here. If you take the FCER or alpha transgenic mice, they express FCER receptors just like a human on dendritic cells. The mouse receptor is knocked out. You take serum from people. You take the serum from the allergic people, and you inject those mice. You sensitize them to human antigen. You could then skin test those mice. You can do PCA on those mice.

The only shortcoming in that system: The mice are small. We need giant mice. They don't have enough skin. But I mean, conceptually we use that for other things, and we use it with human IgE antibody. So you could get a pool. You could have a pool of human Ig serum for ragweed, and you give it to -- you know, there's many things that could be done which would allow an in vivo system that you could put large

numbers through, mice instead of people, just breed them. Just an idea.

CHAIRMAN OWNBY: Are there any other questions that FDA was interested in pertaining to this?

DR. SLATER: Let me just clarify what I've been hearing from the Committee, and please correct me if I'm wrong.

It sounds like the Committee acknowledges the validity of adequately powering studies in order to demonstrate the kinds of bioequivalence that we've been talking about.

It sounds to me like the Committee is somewhat concerned about the consequences of this in terms of how cumbersome the studies might be, and is suggesting that we think about other appropriate measures of overall allergenicity that might escape some of those. But given that we haven't had this workshop yet, and given that we haven't found an alternative method that's been appropriately validated and as a good surrogate for this testing, the Committee is comfortable with the analysis that Dr. Lachenbruch and I discussed.

DR. SAXON: I am comfortable with it, but I must say, I feel some sensitivity. If this was set

up in 1982 -- When did it go in the Federal Register the first time, '94 you said, Jay?

DR. SLATER: November 1994.

DR. SAXON: Yes. I mean, with the concept that it was four to six, and then all of a sudden, you come back eight years later and say, hey, guys, four to six has turned into 100. It don't seem fair, to be frank. It was set up, you know, by good people thinking they had done a good thing, and you may turn it into something burdensome. That's all. You know, that's the concern.

DR. SOTO-AGUILAR: Could 20 individuals per center be all right for a maximum of 100?

DR. SLATER: It's hard to answer that question specifically. It would depend on how many centers, how diverse the centers were from each other, what the population was. I really couldn't answer that specifically. But there's certainly nothing a priori that says that that would not be adequate. We would just have to look at how we are setting it up.

CHAIRMAN OWNBY: Any other comments or discussion on this item? I get the distinct sense there is a consensus that it's lunchtime.

So we will adjourn until 1:50, and we will take up the cloudy issue of particulates in the

allergen extracts.

(Whereupon, the foregoing matter went off the record at 12:48 p.m.)

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

(1:51 p.m.)

CHAIRMAN OWNBY: We will reconvene the Committee meeting, please.

The first item on this afternoon's agenda is a discussion of particulates in allergen extracts, and Ms. Bridgewater is going to present this.

MS. BRIDGEWATER: Thanks. Okay. Good afternoon. I am going to speak to you today about a topic that you have heard about a couple of times this morning, and that is precipitates in allergenic extract.

Before I discuss the precipitates, I will briefly review the product line. There's currently 19 standardized products. The short ragweed product comes in both glycerinated and aqueous formulations. The standardized cat, grasses and mites are generally glycerinated only. There's a few exceptions. Some of the cat pelt extracts come in an aqueous form and the intradermal tests have less than 50 percent glycerine.

Standardized venoms don't enter into this. They are freeze dried with an HSH --

Now the unstandardized products account for the majority of the product line, and they come in both glycerinated and aqueous formulations. I think -

- I was talking to one of our manufacturers this morning, and I think this number is a little high these days. Several of the manufacturers have cut down their product line. Next slide, please.

So historically, precipitates have been recognized for many years, and they are an intrinsic phenomenon. In other words, the precipitates develop within the extract itself as opposed to an external contaminate which would more properly be defined as a particulate.

Now there were early efforts by industry to characterize the precipitates such as trying to describe their physical appearance and look at their solubility properties. Now there were also some early efforts by industry to remove the precipitates.

An example of this would be a manufacturing change, for example, a bulk settling step. This is a procedure where the bulk is allowed to sit for a period of time to allow the precipitant to settle out of solution and then it's filtered, and the manufacturing process continues.

There are also some early efforts in formulation changes, for example, changing the extraction fluids. But these were generally unsuccessful.

So considering that I've told you that precipitates were recognized for many years, how did this issue arise recently? Well, the appearance of precipitates were noted during several Team Biologics inspections in the last few years.

The inspectors observed, physically observed, the precipitates in bulk containers, final containers and retention samples, and they were also found to be a primary cause of customer complaints and product returns.

Can we go back? Sorry. I want to point out from a strictly current good manufacturing practice standpoint that particulates are an undesirable attribute of a parenteral product. For example, the USP sets limits on particulates in parenteral products, and I'm making a distinction here, because the USP specifically talks about particulates. It doesn't really distinguish between particulates and precipitates, as I've defined them.

So here's some of the terms that industry uses to describe the appearance of the precipitate: Crystalline, flaky, cloudy, powdery, tarry, fibrous, clumpy. Next slide, please.

Continuing, they may also describe the color of the precipitate, the ability to resuspend it

in solution or not, the amount of precipitate at the bottom of the vial or perhaps the percentage of extract that contains precipitate.

So I'd like to show you an example of a precipitated short ragweed extract. This is something that was taken from our lab, and you can see here on the bottom of the vial there's clearly a ring of precipitate at the bottom with the clear extract solution on top. Next slide.

This is another example from a different manufacturer, and again this is a short ragweed. This is aqueous. Let me point that out. You can see, this is a little bit different in appearance. It's cloudier, but you can still see this amount of precipitate here at the bottom.

As these two examples illustrate, the appearance can vary widely between extracts, even of the same type. Next slide, please.

So currently the manufacturers -- we've asked them to take some efforts to characterize their precipitates. The first example here, microscopic examination to determine what their physical appearance is; sterility testing to rule out that the precipitates are not a result of microbial contamination; and finally, some of the manufacturers

are doing isoelectric focusing or SDS-PAGE examination of the extracts.

What we've seen from that -- and this is just very initial data -- that in some cases the profiles are similar, and in some cases bands are missing or lose definition. But again, these are for unstandardized extracts. So the relevance is not known yet.

DR. SAXON: Those are incredible examples. I mean, I've been around a long time. I have never seen a vial that had even that ring at the bottom. That other one looked like 80 percent flocculent material.

Do you have any kind of -- I mean, every vial has a little bit of dust, if you shake it. Do you have any idea of like a range of what's really been seen? I mean, so we can get all our ideas around what are we talking about.

MS. BRIDGEWATER: Well, yes, that's a good question. The short ragweed is by far the west offender.

DR. SAXON; At what concentration, for example? When you say is this something at the highest concentration that might be sent out or is this something that we would never see? Do you know?

MS. BRIDGEWATER: No. I mean, this is a standard short ragweed extract. That's a final container vial. So that's how it could be shipped.

DR. SAXON: Incredible. Has anyone ever seen -- Dennis, have you seen any?

CHAIRMAN OWNBY: Yes, I think running a large extract lab, you'll see these occasionally. The question I would have on some of these -- I've seen some that look about as bad as that vial. Though once you bring them to room temperature, it all disappears.

Some of these seem to be very temperature labile. Some of them aren't.

DR. SAXON; I've just never seen any. I mean, I see lots of them a little bit, you know. Actually, it's like a cryocrit. You know, what percent is -- But that one was incredible. Okay.

MS. BRIDGEWATER: Well, I think it really varies. In some extracts you will only see a few flakes in them perhaps, but the short ragweed, like I said, is the worst offender.

DR. LEHRER: Just to pursue that, is this -- do these occur after a certain period of time? Do they occur immediately, and do you see similar amounts with the same preparations or does that vary?

MS. BRIDGEWATER: That is probably a

better question for one of the manufacturers. But, Shirley, did you want to address that? I mean, I think we're going to get to some of that and, if I don't answer your question, remind me. Next slide, please.

Where am I? Okay. So here's some -- There's also some current industry efforts to characterize the precipitates in terms of, for example, looking at the potency of the short ragweed, which we just saw, looking at the potency of the final container extract to the initial value to see if there is a change in potency, and for the nonstandardized extracts looking at the protein nitrogen unit pH and phenol also over the shelf life of the product to see what the effect is. Next slide, please.

So now I'd like to show you some preliminary data that we've gotten. As I said before, with the exception of the short ragweed, all the precipitated extracts are nonstandardized.

So we have to look at some of the other attributes, and these are some preliminary data that we've gotten from manufacturers. So you can see from the first example the penicillium extract, the comparison between the release and retest of the precipitated extract. There's a decrease in the pH

and the PNU.

Then if you look at another example, here this three-weed mix, there's actually an increase in the pH and the PNU. In some cases -- and I picked this example specifically -- the pH dropped actually to where it was out of specs -- excuse me, out of specifications, and that's why this is in red. But again -- and here in this final example, you have a decrease in the pH but an actual increase in the PNU.

So at this point, we don't have a clear picture of what's happening, and we don't have enough data to really say with certainty what the long term effects are. Next slide, please.

So here are some data on potency of standardized short ragweed before and after precipitation. These data were taken from pooled datasets, two sets of 19 lots each.

You can see in this example that for lots A, B and C there was a decrease in the antigen E, which is what we measure for potency, while in the last example -- this is D -- there was an increase.

DR. SAXON: What is pre and post, pre what and post what?

MS. BRIDGEWATER: Pre and post is -- and again this is how the manufacturer sent it to us.

DR. SAXON: Oh, I see. So the manufacturer did this test?

MS. BRIDGEWATER: Right. So the pre is before, I guess, the precipitate either occurred or was removed. That was the value before, and this is the value after.

DR. SAXON: Okay. I thought you did it.

MS. BRIDGEWATER: No, sorry. Let me point out, this is just a snapshot in time also. This is not over the whole shelf life of the product.

So when we looked at the whole dataset of all 38 values, we did notice that there was a five percent drop in antigen E potency, but the difference was not statistically significant.

DR. CLAMAN: These are four different lots from four different manufacturers?

MS. BRIDGEWATER: No. These are actually from the same manufacturer, but they are four different lots.

DR. CLAMAN: So I don't see how you can say it's not statistically -- I mean, it's not fair to pool them.

MS. BRIDGEWATER: These are just -- Well, would you repeat that?

DR. CLAMAN: Well, in Lot C you've got a

drop of 30 percent.

DR. SAXON: An increase.

DR. CLAMAN: I'm sorry. No, see, you've got a decrease of 30 percent.

DR. SAXON: 113 percent.

DR. CLAMAN: No, that's a number.

MS. BRIDGEWATER: Sorry. That's just the absolute. That's not the absolute. That's the value, the antigen E value. That's not a percentage.

DR. CLAMAN: So I don't think it's fair to pool them and say there was no significant change.

MS. BRIDGEWATER: Jay, did you want to respond to that?

DR. SLATER: I think Dr. Claman is saying that he thinks there are significant changes. But on the other hand, this is just four lots. We need to actually look at more.

MS. BRIDGEWATER: Right. And these are four lots that came from 38 values. So it's not a complete picture, obviously. I can't put all 38 up here.

CHAIRMAN OWNBY: Well, it really depends on what we're talking about as a significant change. I think Henry's point is that for extract C it looks like there's certainly a significant loss of potency

in that particular lot, whereas overall you haven't achieved statistical significance across all those lots.

DR. SLATER: Well, I think that's correct, and I think, you know, there are a couple of different ways to analyze it, and that is how many lots lose a significant amount of potency. That's a different way of analyzing it.

MS. BRIDGEWATER: So what's our current state of knowledge about precipitates in extracts? Well, generally we can say that the aqueous extracts precipitate far more than their glycerinated counterparts. As you saw from those pictures, the aqueous short ragweed commonly precipitates.

We know from data we have collected on inspection that precipitates are a primary cause of physician complaints, particularly as a visual appearance complaint, and product returns.

They do not appear to be caused by microbial contamination. On this last point, I think this is something we heard from industry, actually, that the extraction ratio or the concentration of the extracts and possibly the phenol may contribute to the precipitation problem. Next slide, please.

So here's what our current knowledge gaps

are, and these are areas in which we would really like to develop some additional data: The physicochemical composition of the precipitates themselves; the long term effect on the potency of the extracts, and again we are only talking about short ragweed here.

Here's an area that we could really use some discussion on: How precipitated extracts are treated in clinical practice? Are they recognized by the people that are administering the shot, and how are they treated? Are they administered as is? Are they decanted and administered or just returned?

Continuing: Is there a higher risk for adverse events if the precipitate is injected? Now we need to remember, most of our adverse events, of course, come in via the MedWatch system, which is voluntary for physicians and passive. In fact, we receive a very limited number of adverse event reports on allergenic extracts, considering the total number of shots that are administered per year.

We need to remember also that, because they are passively collected, they can't be used to link cause and effect. Rather, the MedWatch system is used as a signal system to identify medical events or areas that need to be actively studied.

Finally, this is a problem frequently for

allergenic extracts. The information submitted is usually incomplete. Many times you don't even have identification of the manufacturer of the product or the lot numbers.

So the current research area that industry is undergoing at the moment: Again, physicochemical composition of the precipitate; some potency instability studies of precipitated standardized; and evaluation of pH, phenol and PNU of precipitated unstandardized extracts over the shelf life of the product.

So our current regulatory position with respect to precipitated extracts is we have asked the manufacturers not to ship final containers in which the precipitates are visible. We have asked them to develop an in-house quality control program to identify and describe the precipitates, validation of any reprocessing procedures that they may perform to remove the precipitate, and right now we are also working on modification of the labeling to address this issue.

Finally, as Mary Malarkey said earlier this morning, we've asked them to submit the biological product deviation reports, formerly known as error and accident reports, on all precipitated

lots to CBER.

Before we move on to the discussion and to the next presentation, let me just summarize the issues. Again, we do know that precipitates occur in allergenic extracts. We know that the aqueous extracts precipitate more than the glycerinated extracts, and following that, almost all of the precipitated extracts are for unstandardized products.

We do have some knowledge gaps on precipitated products, and we are collaborating with industry to fill these gaps. Really, in the absence of data, we've tried to take a prudent regulatory approach to this.

My next slide is the committee discussion points. I wonder -- It might be better if we hear from industry's perspective first before we come back to this. Shirley?

CHAIRMAN OWNBY: Any burning questions, since we asked Ms. Williamson to present a discussion from the APMA point of view? Okay.

MS. WILLIAMSON: Again, I think I introduced myself a little bit earlier, but I'm Shirley Williamson, and I'm representing actually the Allergen Products Manufacturers Association as the president of that organization.

I'd like to take this opportunity to thank Dr. Slater and whoever else was involved for giving us this opportunity to make this presentation.

The first thing, I want to start by saying, as I go through this, you will be sitting there thinking, well, wait a minute, I just heard this. I want to tell you from the outset, Jennifer and I did not know what each other was going to say in our presentations.

You will probably also figure that, if we had collaborated, we would have done a better job of not repeating each other. But I think it does point to the fact that, in fact, industry and CBER, FDA personnel are having discussions about this, working together, and I think this will show we are pretty much working with the same information base as a starting point.

Precipitation for our definition, as Jennifer pointed out, in allergenic extracts refers to the phenomena whereby intrinsic and initially soluble components from the solution become insoluble and form a visibly evident sediment over time.

This contrast was what we refer to as a particulate, which is an extrinsic particle that is present in the solution, generally, I think, of like

a fiber, a piece of paper fiber, a small glass particle, something like that being a particulate.

The history, another review of history: Precipitation has been recognized by the industry as product characteristic for over 30 years. The reason I picked 30 years is that, actually, within the industry there are a couple of individuals that have been with their companies for 30 years, and they remember precipitate being a problem when they started their careers. It probably existed long before that, but that's why we kind of picked the 30 year time point.

Over these years, manufacturers have attempted to characterize or solve the problem of precipitate with very limited progress and even less success.

As you heard earlier, precipitation was raised to a compliance level beginning with the observations that were made on the 483 Forms presented to several manufacturers during their inspections. Resulting from this and concern about what was happening, representatives from several of the manufacturers met with CBER personnel in October of 200 to discuss the issues that were involved.

Types of products: Again, you have heard

some of this, but essentially all types of allergenic extracts reportedly will form precipitate. However, we do tend to see precipitation most commonly in the aqueous or the non-glycerinated extracts, more highly concentrated extracts, the one to 10, the one to 20, 40,000 PNU. But it's also been observed and reported in more dilute forms.

Many pollen extracts will form a precipitate, but it has been noted by some manufacturers anyway that genera do seem to be more prone than others.

Visual appearance: I think Jennifer actually found one more or two more categories than I did, but the precipitate appearance will vary oftentimes, depending upon the product. In some cases it is described as filamentous or like small fibers, stringlike particles in the extract; granular, sandlike, little particles that tend to more settle on the bottom; crystalline which are more angular, and they are generally clear and often they will actually be suspended within the extract; cloudy, which is diffused rather than distinct particles with kind of a hazy appearance; flaky, flat pieces, often have some color or sometimes it's a film, either at the top of the extract or on the bottom.

An interesting thing that has been observed by most manufacturers is that precipitate may appear in one lot of product manufactured from a given lot of source material, and may not be seen in another lot of product manufactured from that same lot of source material, but manufactured under essentially the same manufacturing conditions.

DR. SAXON: If you get it one lot, you get it in all the vials, more or less.

DR. WILLIAMSON: Yes. Now you will see it develop faster. I mean, for some reason, you might see it in one vial and --

DR. SAXON: You might get 1,000 vials, and then you -- and lot -- they will have it, and then the next run-through may not, you are saying?

DR. WILLIAMSON: Correct. One lot of source material may result in several lots of extract that don't form a precipitate, but the next lot of source material, those extracts may.

I think what is important with this is the description, the varied appearance that the precipitate will take on, that sometimes you see it, sometimes you don't, tells me more than probably anything else that -- at least my belief, and I think shared by many of us in the industry -- is it's not a

single phenomenon. There's not a single causative factor involved here.

So that makes the attempt to solve the problem much more difficult, because it probably is a multitude of causative agents.

So characterization analysis of the precipitate: Again, as Jennifer pointed out, one of the things the manufacturers have done is examine these for any relationship to microbial contamination, that being our first and foremost concern.

Some manufacturers use just a microscopic exam of the material. Others actually do sterility tests. But in no instance has a manufacturer reported an association of the precipitate with microbial contamination. It's not to say you couldn't have a precipitated extract become contaminated, but when we have done this direct look-see, it is not microbial contamination.

Some of the major difficulties in characterizing these precipitates result from the fact that, even though -- and you saw the example there that Jennifer had of this gross amount. When you try to collect that material, you are actually surprised at how little you get to be able to do anything with.

Then even if you were able to collect an

amount of this, obviously, you want the precipitate clean if you are going to do any characterization studies because if it is, quote, "contaminated" with the extract, you may be reaching a wrong conclusion. You may say, oh, it contains protein, and in fact you are looking or seeing the protein from the extract.

A number of manufacturers that have attempted to do this have actually found that as you try to clean the extract to -- or the precipitate to remove it from the extract, it redissolves in your cleaning solvent. Then there you are.

A few limited characterization results have been obtained. What little data there are suggest that the precipitates consist of chemicals derived from the source material, such as some polyphenols or flavinoids that will agglomerate proteins or agglomerated protein carbohydrates that form sediment.

In one instance, it was reported that some crystalline appearing precipitate was identified as calcium oxalate. But that pretty much sums it up. There is very little data.

So again a concern for us is what is the effect of precipitate on the suitability of use of these products. Foremost in all of our minds, be it

the industry, be it the FDA, be it the consumer, is the safety.

Again, no controlled studies have been performed, and I'm not even sure exactly how you would want or could do them. But none of the manufacturers have reported any adverse events or patient safety issues associated with the presence of precipitate in extracts.

Jennifer pointed out very clearly in what she said about this that it's not a very good system for saying there is no problem, because oftentimes again the reports are coming from physicians' offices. They may not even be paying attention to the visual characteristics of the extract or in some cases I may be more concerned about the extract that I was using prior to the one that caused the adverse event. That may be where the problem was.

So the point of this is just that we don't have any evidence that says there is a safety issue, but we also acknowledge that the database here is also quite limited.

We have also looked at the potency composition and protein content, as Jennifer mentioned. At the meeting in October, there were some data presented, and there's been a little more data

generated since then.

This again just reiterates -- This information was presented at this meeting in October. Then go to the next one. Standardized products, obviously, would be the best ones to kind of give us a better handle on what is happening to these extracts as they precipitate.

The ironic thing is it's the standardized extracts, at least the grasses and the mites, seem to be the least likely to precipitate. However, one manufacturer did have two lots of Timothy that had precipitated some one to two years after the date of manufacture.

They took a look at the ELISA potency. This was at the time of release. This was -- They reassayed the extract with the precipitate still present. They assayed the supernatant after they centrifuged it to remove the precipitate, and they also assayed the supernatant when the precipitate just settled by gravity.

Again, you can talk and are these numbers different or not. However, all of these values do fall within the limit for labeled potency that these products were labeled at.

Then there's some data on nonstandardized