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FOOD AND DRUG ADMINISTRATION

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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH (CBER)

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ALLERGENIC PRODUCTS ADVISORY COMMITTEE

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MONDAY

MARCH 5, 20001

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The Advisory Committee met in Versailles I and II Rooms in the Holiday Inn Bethesda, 8120 Wisconsin Avenue, Bethesda, Maryland, at 8:30 a.m., Dennis R. Ownby, Chairman, presiding.

PRESENT:

DENNIS R. OWNBY, M.D.	Chairman
HENRY N. CLAMAN, M.D.	Member
SAMUEL B. LEHRER, Ph.D.	Member
DOLORES C. LIBERA	Member
ANDREW SAXON, M.D.	Member
MARIA C. SOTO-AGUILAR, M.D.	Member
DALE T. UMETSU, M.D., Ph.D.	Member
WILLIAM FREAS, Ph.D.	Executive Secretary

I-N-D-E-X

P-R-O-C-E-E-D-I-N-G-S

(8:34 a.m.)

DR. FREAS: Good morning. I would like to welcome everybody here to this, our 17th meeting of the Allergenic Products Advisory Committee.

At this time I normally would go around and introduce the participants on the Advisory Committee. However, those of you who were following the weather over the last few days know that those who are here are not really participants. They are heroes and heroines. So I would like to go around and introduce them, because we sure are indebted to them for braving the weather predictions and arriving here on time.

In the first seat -- I'm going to be starting around the edge of the table -- we have our consumer representative, Ms. Delores Libera, Director of Publications, Allergy and Asthma Networks, and Mothers of Asthmatics, Inc., from Fairfax, Virginia.

In the next seat is Dr. Andrew Saxon, Professor of Medicine, UCLA School of Medicine.

Coming around the table in front of the podium is Dr. Dale Umetsu, Chief, Division of Rheumatology and Clinical Immunology, Stanford University.

Next we have Dr. Maria Soto-Aguilar, rheumatologist in private practice in Hudson, Florida.

Next we have our Chairman, Dr. Dennis Ownby, Professor of Pediatrics, Medical College of Georgia.

Next at the corner of the table we have Dr. Henry Claman, Distinguished Professor of Medicine and Immunology, University of Colorado Health Sciences Center.

We have two FDA participants that are joining us, and they are Ms. Jennifer Bridgewater, actually at the end of the table, who is our consumer safety officer for FDA; and we have Dr. Jay Slater who is Chief, Laboratory of Immunology, again at FDA.

I would now like to read into the official record the conflict of interest statement for this meeting.

The following announcement addresses the conflict of interest issues associated with this meeting of the Allergenic Products Advisory Committee on March 5, 2001.

To determine if any conflicts of interest existed, the agency reviewed and submitted the agenda and all relevant financial interests reported by meeting participants. As a result of this review, the

following disclosures are being made related to the discussions to be held today.

Doctors Gruchalla, Ownby and Saxon have associations with firms that could or appear to be affected by committee discussions. However, in accordance with 18 U.S. Code and Section 2635.502 of the Standards of Conduct, it has been determined that none of these associations is sufficient to warrant the need for a waiver, a written appearance determination or an exclusion.

In the event that the discussions involve other products or firms not already on the agenda for which FDA participants have a financial interest, the participants are aware of the need to exclude themselves from these discussions, and their exclusion will be noted for the public record.

With respect to all other meeting participants, we ask, in the interest of fairness, that you state your name, affiliation, and any current or previous financial involvement with any firm whose product you may wish to comment upon.

So ends the reading of the conflict of interest statement. At this time, the Director of the Office of Vaccines at FDA would like to come and make a few announcements, Dr. Karen Midthune.

Oh, we have just been joined by Dr. >Samuel Lehrer, who is the Research Professor of Medicine, Tulane University Medical Center, and he, I know firsthand, had a lot of trouble getting here. So we really are appreciative that he made it. Thank you very much.

Dr. Midthune.

DR. MIDTHUNE: Good morning, and thank you for braving the predictions of bad weather.

I would like to take this opportunity to mention that we have three exceptional members of our Advisory Committee whose terms ends this coming August. Since we don't at this time plan to have another meeting between now and August that would be a face to face meeting, we would like to take this opportunity to express FDA's gratitude for the dedication and service that these members have provided to the Advisory Committee.

Of course, it's our hope that we will continue to be able to work with these individuals of consultants to the Center in the future. Would Doctors Henry Claman, Andrew Saxon and Dennis Ownby please come to the podium.

First off, we are very thankful to Doctors Claman and Saxon for their four years of service on

the Advisory Committee. During that time, they provided us with meaningful and insightful advice regarding our research program, our grass standardization program, and also recommendations for standardization of other antigens. They also provided strong support for FDA to maintain its standards and distribution program.

You also provided us with advice on expanding our lot release limits and assisted us with implementing our proposed algorithm for the standardization of new antigens. As a token of our appreciation, we've prepared some special plaques commemorating your service to FDA.

First, Dr. Saxon.

DR. SAXON: Thank you very much.

DR. MIDTHUNE: First, I would like to read the letter that Dr. Linda Sudaym, Senior Associate Commissioner, has written:

"Dear Dr. Saxon: I would like to express my deepest appreciation for your efforts and guidance during your term as a member of the Allergenic Products Advisory Committee. The success of this committee's work reinforces our conviction that responsible regulation of consumer products depends greatly on the participation and advice of the entire

health community.

"In recognition of your distinguished service to the Food and Drug Administration, I am pleased to present you with the enclosed certificate. Sincerely yours, Linda Sudaym, Senior Associate Commissioner."

DR. SLATER: Thank you very much.

DR. MIDTHUNE: And now Dr. Claman. I won't read the letter again, but Dr. Claman, of course, got a similar letter from Dr. Linda Sudaym, and here's the plaque.

Now for Dr. Ownby, in addition to your service as a committee member, we are especially thankful to you for your willingness to serve as the Committee Chair and lead us through deliberations from 1998 through the present. Thank you very much for your willingness to perform this public service and for working so closely with us over the years. Thank you very much.

(Applause.)

CHAIRMAN OWNBY: Now it's my turn to say something. I would like to welcome you all here today to these deliberations. We have a somewhat full agenda today, although I think we should be able to get through it without too much trouble, and we will

try to keep everything on time.

I believe our first report is going to be from Dr. Jay Slater, who is going to give us a review of what the laboratory of Immunochemistry has been doing.

DR. SLATER: Thank you, Dr. Ownby. Again, I want to thank everybody for coming out, in spite of the threats of bad weather.

The Laboratory of Immunobiochemistry had a very good year this year. It's been a very busy year, especially over the last several months. What I would do at this point is just review what our staffing is, and many of the staff members are here in the audience, and I am going to ask them to stand when I mention their names.

I am Jay Slater. I'm already standing. I've been here for two and a half years as the Lab Chief. Dr. Lyudmila Soldatova -- please stand -- is a visiting scientists. She has been with the Lab for three and a half years, and we are going to be talking more about her work with bee venom and Hyaluronidase mutants later on.

Melissa Patterson is our Senior Biologist. She has been with us for a year and a half. She has been very busy with cockroach studies over the last

six months, and in addition, during a fairly lengthy period that we were pretty short staffed, she pretty much single handedly kept up the regulatory functions of the Laboratory.

Mona Febus is a microbiologist who has been with us for a year. She came from a different laboratory and has been learning our regulatory techniques very nicely, and really contributing actively to the Lab activities.

William J., or Jonny, Finlay is one of the newest additions to our laboratory. He is a post-doctoral fellow from Ireland, and he is going to be doing some further work with cockroaches that I'll make reference to later on. He came with money that we obtained from the Asthma Initiative last year through the ERDA Fellowship Program.

Marc Alston and Cherry Valerio are our two newest biologists. They are standing back there. Marc and Cherry just joined us a very short time ago, and actually in the short time that they have been with us, they have really picked up the protocols very, very quickly.

They are actively involved in our regulatory functions, and they are -- to my great excitement, they are just getting very much into the

research activities as well. So we are looking forward to them being major contributors to the Laboratory.

Al Gam was a happy inheritance that we obtained from another laboratory. Al is a very experienced biologist in the Division, and actually was closely involved with our laboratory during a period about three or four years ago, helping to get some of our assays into somewhat better shape. Al is joining us, and will be involved with several special projects over the next several years.

Finally, Ron Rabin indeed is our new Senior Staff Fellow. He literally started one week ago today with our lab. He comes from NIH, and will be doing both clinical reviews, medical activities and, in addition, is setting up his laboratory to continue some of the very exciting lymphocyte research that he has been doing as a part of his work at NIH for the past several years.

Beth Cardinale is working part-time with us. She is a biologist who has been working mostly on the Hev b 5 mutation studies.

Gerry Poley is a physician at Children's Hospital who has been a Guest Worker with us doing research a day and a half a week, and Li Shan Hsieh,

Ph.D., used to work in the lab full time. She is now at CDER, but she's been continuing some of her projects in the lab part-time.

This is a graph that I started showing two years ago, and this is a graph of the biologist staffing from 1998 to 2001. You can see here that we are at a very happy state of really being, I think, at a full complement in terms of our biologist staffing.

Just to note again that we went through several months this year, from August until just last month, of being fairly low in terms of our staffing, and this was a period in which Melissa and Mona really bore the full brunt of the regulatory activities on their shoulders, and I think they did a really, really good job, and we really didn't miss a beat during that period.

Among the routine regulatory activities that we are involved in, many of them involved maintaining the U.S. references -- the U.S. standards of potency. Our laboratory develops references, and we will be talking about that with reference to cockroaches shortly.

We also distribute the reference standards to all of the manufacturers. I just want to show you the volume that we do distribute. In 1999 we

distributed 1983 vials in 104 shipments sent to our allergen manufacturers.

That volume has increased. In the year 2000 we sent our nearly 2400 vials in 146 shipments sent to manufacturers. So the trend at least over the last couple of years is we are already sending out quite a large number of shipments and vials, and that has gone up.

We are involved in reference maintenance, and this is actually a very time consuming activity. We check all of our references every six months for potency and for appearance on SDS-PAGE, and we are involved in replacing those references that are either running out because of expiry or just not having enough reagent to continue.

The reference replacement procedure is one that I've shown you before. We identify the references to replace. We select good candidates from recent submissions from the manufacturers. We do initial testing.

I think I sort of give short shrift to this initial testing. We do a lot of initial testing of these products at this point. Then we select a provisional reference replacement.

We send it out to all of the

manufacturers, and ask them to add to our body of information about reference replacement. Then we review the results. We either confirm our original selection or we go back to step 2, depending on the data that the manufacturers sent us.

In the year 2000 we replaced four extracts: sweet vernal, a dust mite serum, an anti Amb a 1 serum, and a dust mite extract. We sent three out of four of these out to the manufacturers for testing. For the sweet vernal, three manufacturers participated by sending us back data. For the anti Amb a 1 five did, and for the dust mite four did.

This is a comparison of the relative potencies of the new and old extracts of those that we have replaced, actually a slightly longer period, since October of 1999, and you can basically see that the potencies have really been maintained fairly tightly, which is what we are looking for.

It's certainly very disruptive to the manufacturers when we change references, and they are not really right on target as to the previous ones. So we do invest quite a bit of effort to try to avoid that from going on.

We are going to be replacing more references in the year 2001. On the basis of volume

remaining, we absolutely have to replace these two, the Anti-Fel d 1 serum and the cat hair.

On the basis of the nominal expiry date, we have another six extracts that we should be replacing and, hopefully, we will be able to replace all of these over the course of the next year.

We are trying to escape from the cycle of having to replace references on an ongoing and frequent basis by lyophilizing the extracts, as I reported to you two years ago, and last year we have started a study in which we are lyophilizing a portion of all of our extracts, and we are going to be following their stability performance over a period of several years, comparing it to the extracts that we have available, and I will be showing you some of that data in the next lecture.

Before we get on to the presentation about the research activities, I just wanted to very briefly note that two guidance documents that you have seen as an Advisory Committee in their pre-draft stages and in their draft stages actually came out this year.

I included those in your packet for you to see them. I did not intend for there to be a lengthy discussion of them, but I do want to note that we do actually come out with guidance documents, and the

good work that the Committee does to help us out does actually come to fruition.

This is the document that we presented to you two years ago regarding the revision of the way we handle lot release for grass and mite allergen extracts in which we tested a slightly broader interval than the manufacturers actually test. That came out in November 2000.

In fact, at the same electronic moment on the Web, this other guidance document came out. This is a document on the stability protocols for standardized grass pollen extracts. This is the document that introduced the concept into those stability studies of using the Bonferroni adjustment in order to correct for the errors that can be built into multiply looking at the same extract over a period of a stability protocol.

Those two both came out in November 2002, and are now being used as our guidance documents for these kinds of features of our program.

That ends the sort of introductory part of our presentation. My next presentation will be the research review, and the next presentation after that will be the cockroach standardization studies that we have done. But first I would be happy to answer any

questions that anyone may have on what I've said so far.

DR. SAXON: Jay, when you replaced like the cat Anti-Fel d 1 serum, how do you do that?

DR. SLATER: The Anti-Fel d 1 serum is a sheep anti-serum.

DR. SAXON: Oh, it's not human.

DR. SLATER: It's not human. That's right. The first thing you do is you obtain a good preparation of Fel d 1. The second thing you do is you find a sheep, and you immunize it, and then having identified an adequate titer, you plasmapherese the animal.

DR. SAXON: It's sheep. That wasn't clear to me, because if it was human, I was wondering where -- You don't go get one of the sheep from Britain, though.

DR. SLATER: I'm sorry?

DR. SAXON: It's another biologic issue we don't want to discuss, hoof and mouth disease. But, okay, I got it. So you just make it sheep.

DR. SLATER: Yes. Any other questions? Shall I proceed?

It's been a very busy year in terms of research in the laboratory. Our drop in the number of

biologists and the census of biologists between August and December did have an effect, though, on some of our research productivity during that period. In spite of that, I hope you will agree that we've really accomplished quite a bit this year.

I have very good, good hopes for next year. This is scheduled to be a very brief presentation. It's really going to be bullets from the research program.

This is a slide that those of you that have been to these meetings before have seen. This is the basic outline of what we are trying to do, the questions that we think it is of validity for a laboratory in our position to attempt to answer and to attempt to address.

The first broad area of interest that we have is the issue of allergen structure and function, and the second broad area of interest that we have is the area of immunomodulation. Everything that we do in the laboratory addresses these two broad areas.

What I am going to do later on at the end of this particular sub-presentation is I am going to show you the specific regulatory purpose of each of those, just so you can have an idea of where we see our research fitting into the regulatory mission of

the laboratory.

So the first issue of allergen structure and function is the stability of lyophilized references, and this is something I referred to at the end of the first presentation.

This summarizes a lot of data, and the basic message I want you to get from this is that, when we lyophilize the references that we have lyophilized so far -- and that's D. pteronyssinus, D. farinae, red top, meadow fescue, and Bermuda grass, and we have also done cat but that's in a different slide -- when we look at it immediately and when we compare the glycerinated product with lyophilized product or with lyophilized product in the presence of mannitol, there is really no significant effect on the relative potency of these products. These are potencies that are measured compared to the current standard.

Reassuringly enough, when we come back at four months, in some cases at eight months or ten months, or even 14 months, we continue to see no significant difference in terms of the overall potency nor do we see any consistent trends over the period of time that we have observed.

Yes, Dr. Lehrer?

DR. LEHRER: I wondered -- It's something that hasn't been addressed generally with lyophilized materials, but it's been our experience that sometimes there are solubility issues after lyophilization, although I think you addressed that in that you showed you change in activity, which I would expect if there were solubility problems. But I wondered, just visually, do you see any differences?

DR. SLATER: Yes. I'm actually glad you brought that up, because I probably wouldn't have in this presentation.

In terms of the -- Let me just go over this and view it another way. One of the reasons to add mannitol to the lyophilized product is a bulking agent. There's a fear that if you have a very small amount of protein, you might lose it in some deep, dark corner of the vial, and mannitol prevents that from happening.

In fact, we have had significant problems with resolubilizing the mannitol containing vials, but we've had no problems at all resolubilizing the vials in which the product was lyophilized just in water.

So far, with the ones that we have done, we have not had a problem with resolubilizing just the lyophilized product.

DR. LEHRER: How and for how long are the lyophilized materials stored?

DR. SLATER: We've been storing our lyophilized materials at minus 20, and we only started this project a little under a year and a half ago. So the longest that we have is -- The longest data point we have is 14 months, but we are going to be carrying this out for years.

DR. LEHRER: Are they stored under desiccation?

DR. SLATER: The vials themselves are packaged in a vacuum, and the vacuum -- One of the features is, is the vacuum maintained over the period of storage. But, yes, they are supposed to be. But, no, the vials themselves are not desiccated. They are not in desiccator containers.

DR. UMETSU: Jay, what do these numbers represent?

DR. SLATER: These numbers are relative potency numbers. So a potency that is equal to the reference standard would be 1.0. However, due to the standard deviation of the test itself, anything between .7 and 1.4 is statistically indistinguishable from 1. So that range would bring your attention down to this vial here, which has a potency of 0.59, and

that actually was a concern to me.

If you notice, this is the meadow fescue that was lyophilized in the presence of mannitol. So I was very interested in what the follow-up potencies were going to be on that. As you can see, they fell right back into line at 8 and 14 months.

It makes you wonder whether, when this study was originally done, there was a problem getting the whole thing back into the solution.

DR. UMETSU: And are there standard deviations for each one?

DR. SLATER: Yes, there are. I didn't put them on this, because I wanted to -- I actually originally had this sort of separated out into the whole -- into each extract preparation individually.

The standard deviation for the test, regardless of the allergen that is being tested, is 0.1375, but that's in log space. So the 95 percent confidence intervals tend to range about plus or minus .25 or something like that.

DR. UMETSU: Because I notice, for example, in the second line at four months it goes from .71 to 1.4.

DR. SLATER: Yes. Now in fact, you know, with this particular test, these two numbers just

about bracket one, one on the low end and one on the high end.

CHAIRMAN OWNBY: Jay, these are all ELISA inhibition potencies?

DR. SLATER: These are all competition ELISA potency tests.

CHAIRMAN OWNBY: And this is -- Again, it's a human pool or an animal?

DR. SLATER: This is with human pooled sera. Now the cat pelt extracts that we lyophilized, we test for Fel d 1, again this time using a sheep anti-serum and the radioimmunodiffusion assay, and we have data from one month, four months, eight months and 15 months.

You can see again that the Fel d 1 content of the two different lyophilized preparations are certainly in range compared to the glycerinated product itself.

We also ran SDS-PAGE gels on all of these. I am not going to show all of the SDS-PAGE gels, simply to point out that in general we see a pattern somewhat similar to this. This is with the red top pollen extract at one month and at eight months. This is glycerinated, lyophilized, and lyophilized in the presence of mannitol, lyophilized glycerinated,

lyophilized and lyophilized in the presence of mannitol.

Almost invariably, what we see is that the major appearance, the overall appearance looks absolutely identical, and there certainly have been no differences in any bands that we could identify as major or important allergens. But in two or three of the extracts we have the kind of pattern that we see here where there appears to be fairly small and subtle bands that are present in the glycerinated product. In other words, the product had never been through the lyophilization, resolubilization process.

That seemed to disappear right up front. So it's not a stability issue. This is an issue of how it handles lyophilization. We have this in two or three of our other extracts as well, where there will be usually one small band of uncertain importance that appears to disappear in the lyophilized resolubilized product.

Again, we will be following this, and certainly this is one of the things that's going to come into our thinking before we decide to adopt lyophilization.

CHAIRMAN OWNBY: Jay, relative to a band like that, if you just did a straight aqueous extract

without the glycerol, does that band stay there or not?

DR. SLATER: I don't know the answer.

The next area of interest within the general topic of allergen structure and function is the question of glycosylation of allergens. Dr. Soldatova has been actively pursuing her studies with bee venom hyaluronidase. She has four mutants at putative glycolation sites that she has been expressing. She also has mutants at active site areas, and her studies really are continuing and going forward quite nicely.

In addition, we are pursuing cockroach studies that I am going to discuss in great detail in the next talk, and those are really aimed toward the establishment of the U.S. standard, but in addition -- and this is the work that I have Jonny Finlay working on -- we are interested in trying to amplify on the work that's been done by other labs in terms of the identification of significant allergens, in view of the fact that most of the current data are based on seroprevalence studies.

Although seroprevalence studies have great strength, they also have some weaknesses as well. Furthermore, I am interested in pursuing with

cockroach allergens a comparison of native and recombinant products, again thinking that down the line we are going to have to face the regulation of recombinant allergens, just as CBER is involved in the regulation of other recombinant proteins.

Jonny is currently working on generating an IgE-specific Fab library, a combinatorial library. He has actually just started on working on that, but our plan is to try to develop that library from several individuals who have demonstrated cockroach allergy and to use this library as a tool for several subsequent studies that we think will be very important for us.

One is for the affinity purification of native allergens. As you know, several cockroach allergens are available now in recombinant form, but the purification of native allergens can be difficult, and we would like to attempt at least using this IgE-specific library to do some affinity purification of native allergens.

We would also like to use the library to help identify important allergens. Although there are quantitative problems with using this kind of library toward that end, we would like to try to identify which allergens are important in these individuals.

We certainly should be able to help identify important epitopes, and most importantly, we would like to use what we learn from these to compare the immunogenicity and allergenicity of native and recombinant cockroach products. This is a project that has literally just gotten off the ground in the last two weeks, and I'm hoping next year to have much more to tell you about it.

Last year I introduced -- Two years ago I introduced our interest in using physicochemical methods to identify and characterize allergens. In particular, we were going to focus on MALDI-TOF, and MALDI-TOF, as those of you that have been at this meeting before heard, is a matrix assisted laser desorption/ionization time-of-flight mass spec.

MALDI-TOF is a technique that can give very precise prints, very precise profiles, of protein mixtures. The protein mixture is embedded in a matrix on the sample plate. The matrix and the protein mixture are bombarded with laser energy that vaporizes the protein and the matrix.

The protein molecules are drawn into a tube by an electric field, and the particles then fly down the tube in free flight, down the tube toward a detector. The time of flight through the flight tube

is a direction function of molecular mass and an inverse function of the ionic charge.

So by measuring the time of flight from ionization to the detector, we can determine the mass/charge ratio with great accuracy.

Last year what I showed you were several MALDI-TOF profiles of bee venom allergens, and the lesson that we learned from the bee venom allergens is that we probably could make a pretty good profile of the bee venom allergens.

I was able to show you several different proteins that were supposed to be present in one venom and not the other, and you could clearly see there the profile was rather different.

This year we looked at ragweed extracts, and we showed once again that you could see Amb a 1 pretty clearly here in the SDS-PAGE, and this is a representative MALDI-TOF of several ragweed MALDIs that we did. You can see very clearly that there is a peak here.

You can also see that there are several other peaks at lower molecular weights, as you can on the SDS-PAGE. But we get a very good degree of precision here, and we are very optimistic about what we will continue to find as we look at some of these

extracts.

So far, we know that we can do this with venoms. We think we can do it with ragweed as well, and we've had quite a bit of difficulty with cat, which was our next target, and we have really, surprisingly, failed to detect Fel d 1 in MALDIs, and we think that we may need to do some further purifications prior to MALDI mass spec analysis with cat, but we will be continuing our studies with cat, and we will be extending our studies to some of the pollens as well.

Again, our objective here is to try to develop some non-immunologically based, some physicochemical methods that are highly reproducible to profile the allergens that we are charged with regulating.

Under immunomodulation, I presented last year some of our LPS work, some of our epitope work. I am actually going to pause for a moment to talk about Ron Rabin's work.

Now Ron Rabin, as I said, just joined the lab a week ago, and he's been involved in important issues such as getting his computers hooked up and getting the phone lines hooked up, and all of these things take a great deal of time. But when he starts

doing his research, which, hopefully, will be very soon, Ron's work has been to look at the role of the chembakine receptors, CXCR-3 and its ligands in secondary lymphoid tissue and in Th-1, Th-2 cd4 T-cell polarization.

He is preparing a manuscript to describe the work that he's been doing over the last several years at NIH, and he is going to continue that work, and his work is really very exciting, as far as our lab is concerned because, first of all, it's integrally related to the immunomodulation work that we do, and yet will really add a completely new dimension to our capabilities and to our expertise. I'm really very happy that he is going to be working with us.

The LPS and the allergen response, you will remember, is a project that I became involved in when I was still at Children's, and it really has continued here, largely because the results have been so interesting, but also because, while I was interested in LPS for one reason while I was at Children's, I'm now interested in another reason being here at FDA.

We know that several allergen extracts, most notably house dust extract but many others, have

endotoxin in them, and one of the questions that really needs to be answered is what is the immunomodulatory role of those components, if any.

We know that LPS is ubiquitous. We know that it elicits broad immunologic effects in mice and in humans. It has some adjuvant activity. It can accentuate both Th1 and Th2 responses.

I won't go through all the data that I presented last year. What we've done this year is we have expanded the study to look at the anatomic specificity of the effects that we have observed. So we studied -- and this was a fairly large and lengthy study in which we looked at four groups of mice that were all given antigen and LPS. So there was no negative control group in this group.

They were all given allergen and LPS. One group was given both of them by the nasal route. Another group was given antigen by the tracheal route and LPS by the nasal route. Another group was given antigen nasally and LPS tracheally, and finally group four received both of them tracheally.

Obviously, they were immunized on a specific regimen. There were several bleeds. We did plethysmography on the mice, and we harvested their organs at the end of the study.

I'm only going to show the really interesting result, and the really interesting result is the plethysmographic data. Plethysmography, for those of you that haven't done it, can be done on mice now by a noninvasive technique. You don't have to cannulate the mice and intubate the mice. You can actually just put them in a chamber.

What you get out of that is a parameter called penh, which is the enhanced pause in their expiration curve. By measuring their enhanced pause, you can get a good measure of what their degree of bronchospasm is. So the higher the penh, the more the bronchospasm.

These were mice that had completed their immunization schedule several weeks before. At time zero they were each given an injection intraperineally of ovalbumin, and then they were put into the box and measured for many hours. This is a six and a half experiment.

All of the mice did very, very well up until just about two hours, and at two hours one group of mice, the group 4 mice that received both allergen and LPS by the tracheal route, began to have some moderate amounts of bronchospasm that worsened over the next half-hour, and then plateaued and remained so

until they were taken out of the box and put back into their cages.

Now all of the mice recovered, and you can see here that the mice in groups 2 and 3 had some slight bronchospasm that developed later, but the bronchospasm that appeared in group 4 was really significantly different from the other mice, especially from that interval of about 120 to 150 minutes out.

Now the other data were also collected, antibody, spleen cell proliferation, all those things. They weren't different in any of the groups. In other words, all of these groups received antigen and LPS. They all had indistinguishable immune responses otherwise except for this, and we are going to be expanding on these studies this year.

DR. CLAMAN: Do you have any idea what the responsible immunoglobulin isotype might be, assuming that it is antibody mediated?

DR. SLATER: We found no differences in the isotype profiles among the four groups. So the answer is no. That doesn't mean that there isn't a difference, but we looked at IgE. We looked at IgG-1, IgG-2A, total IgG. We found no differences in those groups that were measurable, but we were measuring

serum antibody. There may have been local antibody differences.

Beth Cardinale has continued her work on the site directed mutagenesis of Hev b 5. There are three areas based on our previous studies that we are interested in studying in detail. She has successfully obtained three mutants, one mutant at each of these locations.

She has successfully expressed those mutants. So we actually have three working mutant proteins of Hev b 5 that we are going to be continuing studies with.

During the past several months we have expanded on our collaboration with Robyn O'Hehir's group in Melbourne, Australia. We started collaborating with Robyn over two years ago with our studies with Hev b 5, and we have actually published two papers with that group together. So it's been a very productive collaboration so far.

We are now going to expand it around these Hev b 5 mutants. Robyn is going to be splicing our mutants into her expression vector, which is a His-tag vector, and she is going to be expressing them, and we are going to perform the following antibody studies in parallel.

We have a series of human antisera to Hev b 5, a series of mouse monoclonal antibodies to Hev b 5, some rabbit hyperimmune sera that we obtained from Don Beezhold, and Robyn especially will be doing some T-cell stimulation studies on the individuals that she has recruited in Melbourne to study with Hev b 5 allergy.

Well, let me summarize now quickly and just go back over each of the items and tell you what the specific regulatory part of -- role in the program is.

Our studies on the stability of lyophilized extracts, obviously, is tightly bound to our ability to maintain a U.S. reference standard in a timely manner.

Our glycosylation studies, both of the bee venom proteins and of the cockroach proteins, is very closely involved in our guess that we are going to be heavily involved in the regulation of recombinant allergens over the next five to six years.

Our studies with MALDI-TOF and the identification methods is part of our effort to improve lot release for current and future products.

Our epitope studies are intended to help open new immunotherapy options, and our LPS studies

are very important in terms of adjuvant safety and efficacy.

We submitted five abstracts to the Academy meeting that is going to be happening in March, actually in two weeks, in New Orleans. Three of them are being presented as posters, two of them as oral presentations. We also have two other abstracts that appeared in collaboration with other laboratories.

We have six publications from authors in our laboratory in refereed journals from this past year. Two of them that are bolded in top are primarily from our laboratory. This is a paper by Dr. Soldatova and the rest of us on the stability of dust mite allergens in glycerinated extracts, which appeared early in the year in the JACI, and a paper by Rich Pastor and myself on statistical methods for determining equivalent doses of standardized allergens that appeared also early in the year in JACI.

These other four papers are the product of collaborations, both with Dr. O'Hehir's group and with other groups around the country.

We published three reviews this year, I published an article with Jerry Poley in JACI on latent allergy, and then Lyudmila and I published our presentations at the last Paul Ehrlich Symposium as

review articles in those proceedings.

Are there any questions about the research program before I go on to cockroaches?

DR. SAXON: Jay, you used a phased display library to do what?

DR. SLATER: To generate a combinatorial IgE library. So the idea is to develop a library of Fab --

DR. SAXON: IgE Fab?

DR. SLATER: Well, it's going to be -- Yes, that's the idea. There are some questions as to whether you really can do that, but that's what --

DR. SAXON: It's been done twice.

DR. SLATER: Right.

DR. SAXON: One I know in Australia, and I was very concerned when looking at the results with those at what they may have picked up. They think they have picked up -- Basically, they are all excited about what they think are B 1 type antibodies from humans, and I pointed out it looks to me what they have picked up is carbohydrate reactivity and not the clinically relevant antibodies. So it's very tricky.

DR. SLATER: Well, we are aware of the trickiness of that.

DR. SAXON: Good.

DR. SLATER: And in fact, I think it's from Robyn O'Hehir's group that that was done.

DR. SAXON: Well, it's a guy named Collins in Sydney. He's not in Melbourne. But I just think it's very tricky when you -- to find out when you're talking about clinically relevant, because all of this culture activity against carbohydrates, which you are very, very aware of anyway --

DR. SLATER: I agree.

DR. SAXON: I'm sure you will knock it on the head, but it's going to be tough.

DR. SOTO-AGUILAR: I have a question.

DR. SLATER: Yes?

DR. SOTO-AGUILAR: In the LPS study with the mice, you said you harvested the lungs and the spleen at the end of the study.

DR. SLATER: Yes.

DR. SOTO-AGUILAR: Did you see lymphocyte infiltrating the lung or those particularly synthesized with the trachea?

DR. SLATER: Yes. Yes, and actually, the mice that received especially LPS through the trachea had significantly more inflammatory responses when their lungs were harvested. Mind you, the lungs were harvested several weeks after the immunization

program. So these were chronic changes. These were not acute changes.

The mice that received just antigen in the lung with the LPS nasally did not have as much inflammation as the two groups of mice that received LPS in the lung.

DR. SOTO-AGUILAR: So was it a picture of hypersensitivity pneumonitis?

DR. SLATER: No, it was not a picture of hypersensitivity pneumonitis, but it might have been burned out hypersensitivity pneumonitis. It might have been early on hat they had hypersensitivity pneumonitis.

DR. SAXON: Jay, what is your feeling about using LPS in a mouse? It's so different than the human. When she brings an issue up like that, people are getting concerned. I think it's pretty far different. I mean, humans don't have the md-1 comolecule for the tol system, and they are just different. We're not mice.

In many ways, we are, but not in the -- when it comes to the LPS, I'm not sure you are getting a good look at human disease.

DR. SLATER: Well, I think you raise a good point, if you were talking about a B-cell, T-cell

response purely. Clearly, mice have a different B-cell response in this, by a lot, to LPS from humans. But if you look at -- If you look at the effect in vivo in mice on the generation of IgE, this is not a B-cell driven process. This is clearly a T-cell driven process. It doesn't happen in athymic mice. You can't do it. And so --

DR. SAXON: Yes, but that is probably for another reason. I mean, all the works done by Honjo, they take IL-4, LPS and pure B-cells, and they get antibody. It does not happen in humans.

DR. SLATER: So if there were good reasons to believe that the effect that we see with the adjuvancy were a pure B-cell effect, your point would be 100 percent correct. But there's actually a fair amount of evidence that it's not, and --

DR. SAXON: Dave Peden does it in humans and gets interesting results. I'm just not sure they are the same system.

DR. SLATER: Yes. Dave Peden's study of administering it nasally to humans was actually very interesting, and actually correlated pretty neatly with what we are doing.

DR. SAXON: I think as a model it's interesting, because you get away from those questions

of what's relevant in the human, because he does it in the human.

DR. SLATER: Right. I agree with you up to a point.

DR. LEHRER: Jay, not to kick a dead horse, but I just want to touch upon lyophilization again, because I have some real concerns about it, just based on my own experience. I think one has to be cautious when considering adopting that for storage of standards.

Keep in mind that there can be variability in lyophilized vials, too. For example, our experience has been that, if you -- when you are lyophilizing a large batch of material, if you have any differential in temperature, and you might have some slight thawing and then refreezing which can occur, you will see the material looks very different.

You can have white, fluffy material as opposed to brown, crusty material even apart of that, and that material is very insoluble. So, clearly, there can be significant changes. If this occurs, then there can be subtle changes that one may not necessarily pick up that could alter activity.

Lyophilized material picks up water very readily. Maybe it's the high humidity in New Orleans,

but we've actually weighed out lyophilized material on a scale and actually just seen it increasing pretty rapidly.

Now I realize that's not going to be your approach, but nevertheless, one should be careful in terms of someone taking out a sample and then putting it back, taking it out at room temperature and putting it back in the refrigerator or freezer, because those small differences can significant changes.

My last point is that, in my opinion, lyophilized materials can vary with regard to their solubility according to the source. I think that highly soluble extracts from pollens and so on generally will probably work well, but some of the foods, for example, could be a big problem in terms of solubility.

I know we've seen this, and I don't remember what extracts they were. I just can't remember at this point. But it's just a word of caution, that if you are thinking of adopting this for all types of allergenic preparations, you, I think, should try a variety of them first before adopting this.

DR. SLATER: Well, I don't think we are going to adopt it for any one without having

demonstrated with that one particular allergen that it works well, and I think everything that you've said is something that we need to keep in mind as we go forward. In addition, we probably are going to need to replicate our results on multiple lots of the same allergen, just to make sure we get the same kind of consistency. I think every point you made is very important, and we will look at it very carefully.

This is not a change that's going to happen between now and the next Advisory Committee meeting. This is something that's going to take a while to generate enough data. But if we don't start now, we're never going to generate the data.

DR. LEHRER: You're exactly right, and I think that it just -- One of the reasons why I'm making these remarks is because these are long term experiments, and I think -- and I'm sure you are cognizant of all of these issues. Then it won't be a year from now or two years from now that you say, oh, well, we should have tried this.

I think your point is well made that probably it's going to work well for some extracts and for others it may not.

DR. SLATE: And it may not be an option for others. Right.

Cockroach: At last year's Advisory Committee meeting, we talked at great length about how we are going to go forward with standardizing new and different allergens. We talked about some of the standards that we were going to use, some of the criteria we were going to use, and I proposed three possible broad areas that we might standardize, cockroach and two different molds.

There was a considerable amount of very constructive concern by the Committee at that time about how difficult, how inadvisable it might be to go down the road of trying to standardize molds at this point.

In contrast, there was enthusiasm about trying to standardize cockroach allergens. So that's what we have actually gone forward with, and this is work that has gone on since the last Advisory Committee meeting.

Now let's just review very quickly on the standardization of new allergens how we choose new allergens. What are the impact criteria that we are looking for with a candidate allergen?

We want to make sure that, when we standardize an allergen, that we at least have somewhere a stable, preferably lyophilized material,

if possible, for use as a long term reference extract.

We want to have a good idea of what the consistency is of the currently marketed product. We want to -- Ideally, if we have a choice between products, we would like to standardize one that is in widespread use, again just a matter of conserving your resources. If you want to have as much impact as possible, you want to standardize a product that is currently in great use.

Take into consideration the number of manufacturers producing a product. Take into consideration whether it's used just for diagnostics or whether it's used for diagnostics and immunotherapy.

Finally, in a specific evaluation of a specific product, what the public health impact is of the correct diagnosis and/or of adequate treatment.

So this slide is a shameless attempt to get into the record horrible pictures of cockroaches. In fact, the cockroaches at the bottom are the cockroaches that we will be concerned about, German, American and Oriental cockroaches. But these creatures, although they look like they are out of B-movies, really are --

These are the Madagascar hissing

cockroaches, and they really are consuming a real child-size piece of birthday cake here. These are big things, and they actually had them at the Smithsonian some years ago, and the workers would take them out and put them on your shoulder. It was really a wonderful event.

They are called the hissing cockroaches. They do hiss, and if you want have fun at your Website, go ahead in and search for Madagascar hissing cockroaches. There's one site where, if you click a real audio button, you can hear them hissing. I don't suggest that if you have trouble sleeping at night.

Why is cockroach allergy important? Well, cockroaches are ubiquitous. They are all over. Wherever humans live, there are cockroaches. There are sometimes cockroaches in smaller numbers, in greater numbers, but they are there, and they are extremely difficult to control.

If there is one convincing lesson that those of us that have heard the presentations from Peyton Eggleston and others, part of the inner city asthma study, it is that it doesn't matter what environmentally safe or environmentally toxic weapons you throw at cockroaches, the cockroaches come out of it. It seems just fine.

It's very, very difficult to decrease the exposure to cockroach allergens in the human environment.

Thirdly, there are data, especially over the past five or six years, that suggest that cockroaches may, in fact, be connected to inner city asthma.

Finally, due to the good work of several laboratories, there appear to be several cloned allergens for us to work with and for us to study.

So Phase I of the standardization approach that I proposed last year is the laboratory phase, and that's what I am going to report to you now on, in which we develop or adapt preexisting methods for allergen determination, compare the allergen content of different lots.

In many cases, what we are doing here is we are reproducing work that's already been done. Dr. Lehrer did many of these studies several years ago, but we needed to repeat them and to expand them for the purposes of standardizing.

If the commercial products are highly consistent -- in other words, if looking at what's available commercially they all look pretty much alike, then we may wish to reconsider the impact. We

may find that we want to go to other allergens to standardize rather than to standardize these.

Likewise, if the commercial products are all comparable to the best available material -- and of course, best is in quotation marks, because that's a loaded question -- then we may also wish to reconsider impact.

In other words, if we want to get our maximum impact for standardization, we would like to standardize allergens that seem to be in trouble in terms of either their consistency or their overall potency.

So for our preliminary studies we obtained multiple lots of cockroach from all of the manufacturers. The idea was to characterize and compare the extracts and to identify target allergens that we might be interested in.

The immediate goals were to determine the consistency of available U.S. products in terms of protein content, specific allergen content, and overall allergenicity and, hopefully, eventually to determine the best lot release measures.

What do we use as our reference? You have to pick a reference before you can start studies like this, and the reference that you choose is not

necessarily going to be the reference that you end up with. But it turns out that we had large quantities of references that were cleverly named E2-Cg for cockroach German and E2-Ca.

These were lyophilized extracts that CBER had obtained many years ago from one of our manufacturers, and the CBER lab had actually done several studies on them many years ago. So we had a body of data. We had a large quantity of lyophilized product, and we thought we would start studying that.

Now before you ask the question, there was an E1-Ca, and there was an E1-Cg. That was a glycerinated product that we also studied and turned out to be less potent than the E1-Ca and Cg. You will be seeing E1 in some of the other studies, but it's not the standard that we used.

Very attractive about E1-Ca and Cg is that we even had limited skin test data on them from studies that were done at several centers. Let me talk about that.

You are all familiar with the ID_{50} method of determining the potency of a new allergen. This is basically a method in which serial dilution intradermal skin tests are done on a highly sensitive population.

The orthogonal diameters of the erythema responses are measured for each of the dilutions. Some of the erythema responses are plotted against the log of the allergen dose, and these lines in log space are characterized by a slope and an intercept.

Now when you are doing the parallel line bioassay study to compare to extracts, you actually can measure the relative potency as a function of the difference in the intercept. But when you have a new allergen, an uncharacterized allergen, you use the ID_{50} method in which you determine the mean dose at which a 50 millimeter response occurs, and you can average those and then go backwards and determine what the potency is.

Now when these initial studies were done by Dr. Turkeltaub and colleagues many years ago in our laboratory, they found that for several allergens the model D50 was about 14. So allergens with a D50 of 14 were defined as having 100,000 BAU per ml. By rearranging the numbers, you can actually calculate the BAU per ml as $100,000 \times 3^{(D50-14)}$.

Now before I show you the clinical data, these are very limited clinical data, and we are going to actually give a talk later on that will give you an idea of just how many study subjects you actually need

to do a statistically valid study, but based on the limited data that we have, we can draw some limited conclusions.

These are studies that were done between 1995 and 1998 in four different centers in the United States. Some 20 to 25 individuals were recruited, and out of them we only really got valid data on ten or 11 of them, depending on the extract.

To you look at the German cockroach extract, there were 11 individuals on whom there was valid data. Now these data are stratified in two different ways. On line 1 of each of these two tables, there is a sigma E of zero.

That means that all these individuals were screened with a prick test, and that means that this is all of the study subjects in whom valid data was obtained without regard to what their initial level of sensitivity is. In other words, all comers that came to the study are included in line one.

In line 2, however, we apply a certain exclusion standard. If your initial sigma E is not above 50 millimeters, you are excluded. So this second line would tend to be the more hypersensitive individuals.

Nonetheless, if you look at the D50 that

was calculated for these groups, for the German cockroach it's 11.2 for all comers, and for the more allergic individuals it's slightly higher, say 11.9. If you convert that into BAU/mL, you get something between 4,000 and 9,000 BAU per mL.

Now look at the 95 percent confidence interval. Okay. It's huge. Why is it huge? Well, it's huge because n is very small. You need more study subjects in this kind of study in order to get a tighter interval. We are heading in that direction, but we're not there yet.

What you can say is that this German extract product is not super potent. This is not a 100,000 BAU per mL product or at least it doesn't seem to be, based on these initial studies.

The American cockroach, likewise, interestingly, whether we used all comers or whether we excluded the bottom three individuals and only looked at the seven most hypersensitive individuals, we came out with essentially the same D50, again 16-1700 BAU per mL.

So the take-home message from this is we don't know a lot about these extracts, but we don't think they are very hot.

We obtained cockroach extracts from all

the manufacturers. We obtained 26 extracts. Six of them were aqueous. Twenty of them were glycerinated. They are widely varied in their appearance. The three on the left are aqueous extracts. The one on the right is a glycerinated extract.

I show this really to show you the vast difference in color and appearance. You won't be surprised to learn that vial number 14 is a one to 1,000 aqueous extract, but I am also showing this as a sort of a preamble to a talk that Jennifer Bridgewater is going to be giving this afternoon.

This little darkness at the bottom here, this orange color, is not a photograph artifact nor is the cloudiness in this extract. These extracts both have precipitates in them, which was a fairly common finding among our cockroach extracts, the aqueous cockroach extracts in our study.

Interestingly, even though this glycerinated material looks much darker and richer and you could easily imagine a lot more cockroach stuff in this, this was completely in solution. There was no precipitate in this one.

We looked at all of these extracts using various protein assays, SDS-PAGE, various kinds of ELISAs and Western Blots. So here's the protein

concentration data using the ninhydrin method.

The bottom line on this is that the concentration of the glycerinated American cockroach extracts was about 9 milligrams per mL with a range of about 3.5 to 21, German glycerinated extracts about 4.5 with a range of 2.5 to 10.5.

Now the aqueous data looked horrible in terms of the range. It's about 6.8 mean, but the range is 0.09 to 24.82. This is a little bit of an unfair representation of the aqueous extracts, because while the glycerinated extracts are either 1 to 10 or 1 to 20, the aqueous ones go down to 1 to 1,000.

So don't get too -- There are lots of reasons to say things about aqueous extracts that suggest that they are not as good as glycerinated extracts, but this isn't one of them. This is simply a matter of the labeled dilution of the extract.

DR. LEHRER: Jay, just a quick question. For your protein assay, what do you use as the standard?

DR. SLATER: Bovine albumin. So it's an estimate. Is there a standard you would think would be more appropriate for insect proteins?

DR. LEHRER: No. It's always a concern, because any value is really artificial based on the

protein that you use, and there are just variabilities. But you're right. I think there are going to be issues with any standard, and it's really just a reference to give you an idea.

DR. SLATER: But one thing to remember then -- So remember our numbers were about 4 to 8 milligrams per mL for the glycerinated extracts. Now the E2 standards that we used were considerably higher. They were 25 to 30 by the ninhydrin assay and, therefore, we scaled up the allergen levels and the relative potencies to the reference protein concentration.

I didn't want to just measure this protein difference over and over and over again. So we scaled up the numbers for the allergen levels and the relative potencies. You'll see that in the data. You'll be able to see that we've done that, but I just want to explain why we're doing that.

We revisited the question of which protein assay is best and which ones are interfered with. We came up with very familiar answers. When we look at the correlation of the protein assays to each other, we find a fairly good correlation among the aqueous extracts among all the different assays, r^2 of 99, 95 and 92. So not too bad.

When we look at the glycerinated extracts, things fall apart, and the correlation especially between the ninhydrin and the Bradford assay and the BCA and Bradford assay, and even the ninhydrin versus the BCA assay is really not well correlated at all. Again, this is not particularly surprising.

We know from work that Paul Richman did when he was part of the allergenic products laboratory that glycerine interferes with the Bradford assay and, to a lesser degree, with the BCA assay; and it doesn't appear to interfere with the ninhydrin assay.

So, really, this part of our study really validated the value of the ninhydrin assay as being a good protein assay when you are looking at glycerinated extracts. It's not interfered with. So for the rest of this study, we used the ninhydrin protein concentrations for the remainder of our analysis.

Again, our conclusions were that the protein concentrations of the commercial extracts vary widely, and are lower than the protein concentrations of our references, and again that glycerin interferes with the Bradford and, to a lesser degree, with the BCA assays.

So now we're going to start to look at

some of the specific allergens, and listed here is a listing of the different relevant allergens in cockroach, Bla g 1, 2, 4, 5, and 6, and an American cockroach Per a 1, Per a 3, and Per a 7.

This is an SDS polyacrylamide gel of our various reference extracts. Shown on the left are the German cockroach extracts and on the right are the American cockroach extracts. You can identify several bands that you might think, based on their molecular mobility, might be some of the identified bands.

Needless to say, this pattern is a little bit too complicated to identify this band specifically as Bla g 2 or this band specifically as Bla g 1, but you do get a good sort of rich pattern of specific bands that you can further study.

Likewise when we look at -- I'm sorry. Oh, when we look at the specific commercial products of German cockroach, we come up with some interesting observations. The way this is organized is that multiple lots from manufacturers are grouped together. So the first two columns are two different lots from one manufacturer. The next two lanes are two different lots from another manufacturer.

This fifth lane is from one manufacturer, and these next two lanes are again two different lots

from one manufacturer. What you see is within a manufacturer you have a pretty decent amount of consistency between the two products, but among the manufacturers you see a lot of heterogeneity.

Again, this is an observation that, I think, has been made before. Again, Dr. Lehrer's studies from the past looked at comparisons of different manufacturers' products. But you can see specific bands that are present, major bands that are present for some manufacturers that seem to be almost completely absent for others, and vice versa.

Looking at the commercial glycerinated American cockroach products -- Incidentally, the reason I am not showing the aqueous products is that there's not much to look at. When you do the aqueous products on SDS-PAGE, you don't see much at all. So you do see something. It's not completely gone, but there's just not that much there.

The glycerinated American cockroach products: Again, there's a lot of heterogeneity between the manufacturers. But for instance, these two -- and actually, even these two lanes, which are from the same manufacturer, there's really a big difference here in these bands.

Again, are these specific bands important?

We don't know, but there's certainly evidence here of heterogeneity.

Now one of the things that bothered us was that all of these commercial products and our re-reference standards had a significant number of bands up in the region above 75 kilo-Daltons. One of the things you always worry about with allergen extracts is whether you have aggregates present in those or whether you are really looking at well defined individual proteins.

So we did some studies looking at these gels in the presence of 4 mole urea. Now you all know that SDS, which is used as our denaturant in SDS polyacrylamide gels, is a strong denaturant, and also it has the additional benefit of conferring uniform charge density on the proteins so that they can be separated according to their molecular mass rather than mass and charge.

Four mole urea appears to be a stronger denaturant than SDS, but in our case we used the urea in addition to SDS, because we still wanted to have that charge density uniformity so that we could read the gels more easily.

What you see here on the left is a repeat of the German and American cockroach standard that we

had using a regular SDS-PAGE gel. The same products run concurrently in an SDS-PAGE gel that contained a significant amount of urea, 4 mole urea in the gel, and actually I think there were six or eight mole urea in the sample buffer itself.

What you see here -- and since the markers migrated differently as well, I put these blue lines in to sort of guide you through it. The lower blue line represents the 35 kilo-Dalton standard, and the upper blue line represents the 75 kilo-Dalton standard.

What you see here is that, when you go to the urea gels, the products -- the bands that are above 75 kilo-Daltons disappear entirely, and the bands that are -- even the bands that are between 35 and 75 kilo-Daltons, while they don't disappear entirely, they go away a lot, and you see a real enrichment of the bands that appear below 35 kilo-Daltons.

So we certainly seem to see some evidence here that a lot of the higher molecular weight bands that have been observed before may well be aggregates of lower molecular weight proteins. Well, that's actually good news and bad news.

It's good news, because it suggests that

the list of allergens that we are looking at, which are, for the most part, lower molecular weight proteins, may in fact be a list that is, if not complete, at least close to complete. At least we have a reasonable number of those.

It's bad news, because urea gels are harder to run, but I suspect we are going to have to end up doing that for a good portion of our studies.

Just for interest's sake, since we were pouring urea gels, we actually ran some recombinant cockroach proteins on urea gels, just to see if they migrated substantially differently. So this is a regular SDS-PAGE gel, and this is a urea gel run concurrently.

What you see here-- this is recombinant Bla g 5, recombinant Bla g 4 and recombinant Bla g 2, in the same order, recombinant Bla g 5, Bla g 4, and Bla g 2. So if you look at recombinant Bla g 2, it migrates at about 50 kilo-Daltons in the SDS-PAGE gel, and it migrates just at about 50 kilo-Daltons in the urea gel. So that's reassuring.

Let's skip over to Bla g 5. It migrates at about 25 kD in the SDS-PAGE gel. It migrates a little bit faster in the urea gel, say at about 23 or 22 kilo-Daltons by apparent molecular mass.

So that's not wildly different, and that's not terribly surprising. But, gee, look what happens to the recombinant Bla g 4 that we got. This migrated at 30 kilo-Daltons as, for the most part, a nomomer. There was a small looked like a contaminant peptide here. This is in the SDS-PAGE gel. But it almost completely disappears in the urea gel, but if you look carefully, you see there's a band at 16 and a band at 14 kilo-Daltons. It almost looks as though it's a heterodimer.

Now that's not what is described, and I'm not about to take this and publish it as evidence that recombinant Bla g 4 is a heterodimer, but it certainly is something that we were surprised to see, and we are going to be looking at it some more.

Back to our commercial products, we performed immunoblots using pooled allergic sera of our different products, of both American and German products. For the purpose of time and efficiency, I am only showing the German immunoblot here.

You can just see that there are a lot of different proteins that seem to light up, which suggests to us, at least pooled allergic sera does recognize several peptides in both of these products, and again with specific anti-Bla g 1 and specific

anti-Bla g 2 that we obtained from Endo Biotechnologies, you see a sort of a different picture, depending on what you are looking at.

In the blot using the anti-Bla g 1 monoclonal antibody, we see a band, a prominent band, at less than 10 kilo-Daltons and another band that's somewhere between 35 and 50 kilo-Daltons. Now Bla g 1 is supposed to be between 20 and 25. So we're not really sure what we are seeing here, but the migration of some of these does go differently, depending on the conditions under which they are run.

Now with the anti-Bla g 2 monoclonal antibody, that's somewhat more reassuring. The predominant band is at 36 kilo-Dalton right here, which is what you would expect to see in terms of the migration of Bla g 2.

There is another -- a larger protein of about 80 kilo-Daltons, and we are not sure what that is, whether that is a dimer of Bla g 2 or whether that's another protein that cross-reacts.

Okay. Let's get down to the major business, and that is looking at the relative potency of the cockroach extracts. This was measured by competition ELISA using our pooled allergic serum, which consists of serum from 16 individuals allergic

to cockroaches, both clinically and by skin testing.

What you see here in this column is the relative potency data of the glycerinated American cockroach, glycerinated German cockroach and the aqueous cockroach. And they are all pretty low, .07, .05 and zero.

So just to orient you, a relative potency of .05 means that within the statistical deviation of the study it has about 120th of the potency of the reference material. Remember, too, our reference material is not all that potent. Okay? Skin testing that I showed early on.

Now when we scale it for the amount of protein there, interestingly, the American cockroach doesn't change all that much. The German cockroach mean does increase to .22 with a range of .01 to .46. So one or two of the German cockroach products actually came to something within 30 to 50 percent of the potency of the reference German product, E2-Cg. The aqueous products are all just about at zero by this testing.

We also did specific allergen assays for Bla g 1 and Bla g 2 using monoclonal antibodies. These were actually done for us by Endo Biotechnologies, and the results there were sort of

interesting.

Again, this would only be done for German cockroach. You wouldn't expect American cockroach to have much Bla g 1 or Bla g 2. The mean scaled up Bla g 1 level for the various commercial products that we had was about 3500 units per mL with a range of 2200 to 4800.

Now just by way of comparison, E2-Cg had significantly more Bla g 1, 13,800 units per mL. Interestingly, Bla g 2 -- it was just the opposite story. There was a wide variation in the amount of Bla g 2 present in our commercial products with a mean of about 43,000 units per ml, but the variation was from 8000 to 66000 units, depending on the particular product. But look at where our E2-Cg came out with the Bla g 2 level. It was really on the low end.

So, interestingly, although the products seemed to have significantly lower potency and, certainly, at least a moderately lower amount of Bla g 1, they don't have very much Bla g 2. They have more Bla g 2 than our reference product does, in spite of those other findings.

Just to look at the relationship of all of the different studies that we did, we found a very good correlation of protein concentration and relative

potency, a very good correlation of protein concentration of Bla g 1 levels, and an excellent correlation of Bla g 1 levels and relative potency. And Bla g 2 levels are sort of left out in the dark here. They really don't correlate well with either potency or Bla g 1 levels or protein concentration.

Now one word of warning about interpreting these data. The lack of correlation between Bla g 2 levels and the relative potency could be completely artifactual. Remember, I showed you that our reference standard doesn't have very much Bla g 2 in it.

So even if the individuals that constitute our pool, our serum pool, have a lot of antibody directed to Bla g 2, we are not going to put Bla g 2 in the relative potency equation, because our standard doesn't contain much of it. In other words, the stuff that we are coating with doesn't contain very much Bla g 2.

Therefore, the absence of this arrow here, the absence of a correlation between the potency and the Bla g 2 level, may be an indication of a problem that we have with our reference standard. That being said, the lack of correlation between Bla g 1 and Bla g 2 levels and protein concentration and Bla g 2

levels is not a victim of that particular artifact.

So our conclusions: Commercially available cockroach allergen extracts vary widely in protein content, Bla g 2 content, SDS-PAGE banding patterns, and overall allergenicity. They appear to be less potent and to contain less Bla g 1 than the candidate reference extracts.

So where do we go from here? Clearly, our next stage is to go back into study subjects that are cockroach allergic to determine by extensive IDEAL skin testing the best in vitro potency measures, and presumably the selection of a reference standard, which as I said at the beginning, may not be what we started out with so far. We may go with something else.

Just finally, as a way of acknowledgment, all of the experiments done here were done by Melissa Patterson. Again, during that period in which we were relatively short staffed, she really did a terrific job. Jonny Finlay, who just came on board a short time ago, has contributed to these studies as well, and we are hoping that all three of us will be continuing to do good studies in cockroach over the next year.

Thank you. Any questions about cockroach?

DR. LEHRER: Jay, you mentioned that these were standards that had been in storage for sometime that you were using for the study.

DR. SLATER: Yes.

DR. LEHRER: Do you have any concerns about the stability of your standards?

DR. SLATER: Well, I have concerns, but I can't address the concerns. In other words --

DR. LEHRER: But could you have a fresh extract, for example?

DR. SLATER: Yes. I think that's something that we are going to need to do. Absolutely. That's one of the things that Jonny is actually going to be helping us with, is making fresh extracts.

We have actually obtained some fresh cockroach material which we are going to start working with very shortly.

DR. LEHRER: The other point is with cockroach a major problem, as with a lot of fungal extracts that Bob Esch demonstrated, I think, very elegantly, is the high levels of protease enzymes.

DR. SLATER: Yes.

DR. LEHRER: And this can have substantial impact on stability, not only relative to the issues

that you are addressing here, but also a concern I would have is use in the clinic, you know, of taking these extracts out and putting them back in.

I don't know if that's something that you can address, but it might be a consideration as you look into stability studies later on.

DR. SLATER: Thank you.

DR. SOTO-AGUILAR: I understand they used to prepare them in formaldehyde precipitated products.

DR. SLATER: I'm sorry?

DR. SOTO-AGUILAR: I don't know if they are still doing those, you know, with formaldehyde precipitation. That does contain enzymes, while the right products are freer of enzymes and contaminants. Which ones are being produced right now?

DR. SLATER: Which allergen extracts are being produced?

DR. SOTO-AGUILAR: Right.

DR. SLATER: They are not formaldehyde precipitated products. These are either aqueous products that are just extracted in aqueous solutions of various sorts or they are glycerinated products in somewhere close to 30 to 50 percent glycerine.

DR. SOTO-AGUILAR: Have you recognized any enzymatic products in there?

DR. SLATER: I'm sorry?

DR. SOTO-AGUILAR: Have you recognized any enzymatic products?

DR. SLATER: I haven't looked.

MS. LIBERA: When extracts are finally sent out for commercial use, how are they labeled? Are they labeled with the actual potency or are they labeled with -- I mean, how are they labeled?

DR. SLATER: These are non-standardized products. So these are products that bear the label that there is no U.S. standard of potency. The labeling of non-standardized product can be one of -- typically is one of two ways.

One way is by the dilution, which simply indicates really the manufacturing technique, a one to 10 dilution, one to 20 dilution, one to 1,000 dilution, or it can have a measure of the protein content on it by PNUs or protein-nitrogen units per ml.

When those measures have been compared to actual potency measures, which is what we are trying to aim for here, actual potency measures, they have fallen short. In other words, within a narrow dilution range, there will be a very broad range of potencies or within a relatively narrow PNU range,

there will be a broad range of potencies.

So the correlation between the labeled measures of the non-standardized extracts -- the quantification of those doesn't correlate well with the quantification of actual potency measures.

So these products were all labeled by dilution, one to 10, one to 20, one to 1,000. And, you know, between the one to 10 and one to 1,000, there's actually a pretty good correlation. One to 1,000 has a lot less stuff in it than the one to 10.

The real question is, among products that are labeled one to 10, how close are they to each other in terms of potency? The answer is they don't seem to be all that close.

MS. LIBERA: Okay.

DR. SLATER: Did I answer the question?

MS. LIBERA: Is it confusing for a physician to know then if something is actually working with which patients?

DR. SLATER: Well, one of the advantages of standardization is to give some kind of comprehendible, uniform unitage that a physician can use, not only to compare the same product from different manufacturers, but also to compare different products.

In other words, the unitage is really based on the size of the skin test reaction that it will illicit in a highly sensitive population of individuals. So there is at least some -- One of the great advantages of standardized products is that the unitage, once you get used to it, is really fairly straightforward to understand. The unitage of non-standardized products is not quite as informative and can be confusing. That's one of the problems.

DR. CLAMAN: Yes, it is confusing. And also, if your one to 1,000 doesn't have less than your one to 100, you're in real trouble.

What are the practical -- this is in the same line. What are the practical consequences of your findings? What should the practicing physician do with regard to cockroach diagnostics and cockroach immunotherapy in the light of what you've just told us?

I mean, this is, obviously, a very difficult area of biochemistry dealing with complex materials and having to ultimately rely on bioassays as well as biochemical tests. What should the practicing physician do, if anything, with regard to your findings, if the practicing physician knows about them?

DR. SLATER: The practicing physician should immediately try to encourage the FDA to go forward with its standardization efforts of cockroaches, should encourage the manufacturers to fully participate in that process, and to help us go forward with standardization of cockroach allergens.

I think to give the practicing physician guidelines on dosing or appropriate usage of a non-standardized group of extracts is simply not possible. I can't give guidelines based on this, because this is a study based on looking at 26 different lots.

We don't have reason to believe that our specific quantitative conclusions are applicable to the far greater population of lots of German and American cockroach that are out there. So I can't give specific applied conclusions for practicing physicians other than the practicing physician should understand that standardization is what really makes their job substantially easier in terms of knowing what dose is to be used and what a label means.

CHAIRMAN OWNBY: But, Jay, against your initial criteria that you set out as you started this pilot, you said, one, you wanted to know whether the current materials were consistent between manufacturers. I think you would suggest that that's

not true. There's wide variability between current manufacturers.

DR. SLATER: Right.

CHAIRMAN OWNBY: And the second was whether the current materials are potent or not. By the admittedly limited skin test data you currently have with your standard compared to these materials, you would say they are not very potent, far less potent than we would like to have.

DR. SLATER: Right. So in case I wasn't clear, by the impact criteria that we discussed last year and that I laid out at the beginning of this talk, it's clear that cockroach is a good target for standardization, and we should be going forward with it, by all criteria.

DR. SOTO-AGUILAR: With the test method, the ID_{50} EAL, you are measuring basically the erythema reaction, while the Europeans used the wheal measurement. Are you looking for work comparing the result on the same study population and see how they correlate -- the two methods will correlate?

DR. SLATER: Well, we know how the two methods correlate. We know how intradermal and erythema reactions correlate. They do correlate. The reason that erythema is advantageous over intradermal

is that you get a better slope.

In other words, with intradermal you'll get a flatter slope. You'll get a more consistent --

CHAIRMAN OWNBY: You said intradermal, meaning wheal.

DR. SLATER: What I meant was -- thank you -- with the wheal reaction you get a flatter slope, and it's harder to get good comparative data among products, and it's harder to identify where the right ID_{50} is, because there's an error associated with all of these measurements.

So if you are working in a flat slope and you've got this big error, it's impossible to know whether the ID_{50} is here or here; whereas, if you have a steeper slope, which is what you have with the erythema, even with the same error you can make a pretty guess at where the D_{50} actually is.

DR. SOTO-AGUILAR: I'd like to make a little comment. I'm sorry about the time. But I was involved in the studies with cockroach in '95 and '96, and as I remember, this is in a highly atopic population in Mobile, Alabama. We saw differences between the Caucasian and the African American population where there were different epitopes apparently recognized with the two different

populations. So are you looking at that possibility, too?

DR. SLATER: Well, we have to look at that possibility. One of the reasons that you need to do a large ID_{50} study is you need to get a population that is both geographically and ethnically diverse. Certainly, from what you said, it makes it especially important for cockroach, but we would do that in any case, just because that's one of the things that we need to find out.

DR. LEHRER Can you get an ethnically diverse population with the erythema assay?

DR. SLATER: Well, there are limits on how ethnically -- You know, there certainly are some limits with the erythema assay, but you can. You can. I think it makes it more difficult, but it doesn't make it impossible.

CHAIRMAN OWNBY: Okay. Jay, why don't we move on, and we'll try to take the next report and just have our break a little late.

DR. SLATER: Okay. Let me introduce -- Mary Malarkey is the Director of the Division of Case Management in the Office of Compliance and Biologics Quality, and she will be giving us a compliance update on allergenic products.

MS. MALARKEY: Can everyone hear me? All right.

Well, good morning. It's a pleasure to be here to present the Committee an update of the compliance status of the allergenics industry. As Jay said, I am with the Division of Case Management in our Office of Compliance and Biologics Quality.

When an inspection is performed of an allergenics manufacturer or any of our manufacturers and it is felt that significant deviations from the law and applicable regulations have been recognized, the case comes to our Division for further evaluation to determine whether additional action needs to be taken.

So today, briefly, I would like to go over some of the enforcement actions that we have done, kind of go through what we mean by enforcement actions, and which have been taken against this industry, what we call biological product deviation reports, used to be called errors and accidents, recalls, issues leading to the enforcement actions that Ill mention, and finally some forward thinking in this area.

Please, if you have any questions at anytime, don't hesitate to interrupt me.

In addition, there are a couple of mistakes on my slides. I apologize, but I'll point those out as we go along.

So starting with enforcement actions, this is a listing of those that we normally use. There are others, but these are what are used by all the FDA. The first two are legal actions, meaning that we would have to go in front of a court to take them.

Seizures are when we feel a product is a danger to health. We will ask a judge to allow us to seize the product. U.S. Marshals are actually sent in to do so. Injunctions are taken against firms that have a continuing history of compliance problems. They are again a legal action, and I'm happy to report that neither of these actions have been taken in the allergenics industry.

The rest of these actions are what we call administrative. That is, FDA can take these actions on our own with just legal counsel, internal legal counsel. Warning letters are exactly what they sound like. They are a warning to the manufacturer that they are out of compliance with the applicable regulations and the law, and it's an opportunity for them to address the issues and move on, basically, without us taking any further action.

Unique to biologics is the fact that we have a license. Each one of these products, the standardized extracts, are all separately licensed. The non-standardized fall under one license. But with this we have tools that we can use.

If we really feel there is a danger to health, we can suspend the license, and this means that the manufacturer can no longer distribute the product and is basically shut down. Unfortunately, some of these actions have been taken against this industry, but I'm happy to report that none in recent years.

License revocation is just that. It's actually taking the license away. We don't usually go to this unless a firm requests it under suspension. Normally, we send what we call a notice of intent to revoke, and this is where, for example, after we've sent a warning letter and we go back out and the firm has to taken things seriously, if you will, or hasn't been able to correct the deficiencies, we will proceed to the notice of intent to revoke.

This actually puts the firm under a corrective action plan and gives them an opportunity to come back into compliance. We have other actions such as sending letters after further inspections

under the notice of intent or often we will ask the firm to come in and speak to us face to face, because often that helps get them on the right track.

This is kind of a rundown of the enforcement actions that we have taken with regard to this industry from 1997 to the present time. I'll highlight the mistake in this slide. In 1999 there were actually two notices of intent to revoke that were issued that are not reflected on this slide.

It's interesting to note, if you've heard of Team Biologics -- and I think most people have these days -- Team Biologics took over the inspections of this industry in 1999. So as you can see, many of the issues were first identified by CBER inspectors as well as those from the ORE district offices, and we have seen what we hope to continue to see, a decline.

Now this kind of breaks it off into enforcement versus the inspections that were done. So it gives you a better idea of percentages. I've included here our other major industries, the plasma derivatives, the in vitro diagnostics -- that is, the viral marker test kits for our blood supply, for example -- therapeutics which are namely biotech, our vaccines, and finally the allergenics.

So in fiscal year 1999, 25 percent of the

inspections of the allergenics industry resulted in -- Oh, I'm sorry. That's a mistake as well. It's 37.5 percent because of the additional notices of intent to revoke on my first slide.

Unfortunately, in fiscal year 2000 there was an increase from the number of inspections to the number of enforcement actions, up to 42 percent. Finally, for this year I need to make a couple of notes. Our fiscal years, of course, begin in October. The numbers I have taken are from October to the middle of February.

Given that, if an inspection occurred in fiscal year 2000 but action was taken this year, I included the inspection number under this year's numbers to make the numbers more meaningful. Also, we have other inspections that are either under review in our office or have not been classified.

Now what we mean by classified is, again, when the investigator feels that there's a problem, he will make it official action indicated, he or she, and this will then come to us. But there are other classifications as well: No action indicated, when the compliance looks good, and voluntary action indicated when there are some misuse, but it's felt they can be resolved without further action taken.

So given those caveats, this is this year so far. As you can see, we've done seven inspections and taken two actions, which comes out now to 28.5 percent. That is a decrease, but again it's preliminary data. So we really can't -- I wouldn't want to say anything. I hope this holds out as the year progresses, but this is about five months in.

Moving to biological product deviations, under Title 21 of the Code of Federal Regulations, Section 600.14, it is required that manufacturers report deviations that occur during manufacturing that may affect product that is already in distribution.

These used to be called errors and accidents, but now we have a new regulation. We call them BPDs or BPDRs for reporting, and this final rule, we hope, will be effective in July. Most firms are already adopting it. It's a much better system. There's a form that can be used. It can be submitted electronically. So we are trying to streamline the process.

In regard to this industry, the problems that we have seen: Of course, we have talked about precipitates, and I think Jennifer will be speaking about them later, and there's also stability failures that may result in the need to report.

This is again a three-year history of all of our manufacturers. As you can see, there is a steady increase in allergenics. There's also a bit of an increase in the year 2000 for just about everyone, and this is mainly because our manufacturers weren't reporting under the regulation.

The blood and plasma industry was used to it, but our drug and device manufacturers were not. So this is why -- partially why we have seen an increase. But in the area of allergenics, really, the precipitates account for the high numbers that we see, and this year I believe there's been 81 -- there have ben 81 reports so far.

Now in October we had discussions with APMA on this issue, and industry asked if there was some way they could combine these reports to make it less burdensome. As it stood, or as it stands, they are reporting each lot that is affected as a separate report.

We concur that this is burdensome. It's burdensome both on the industry and on FDA. So we have determined that the reports may be combined. There is a 45-day reporting requirement. So 45 days after a BPDR is discovered, it must be reported, but again these can be combined now. So, hopefully, that

will help matters and burden to industry and FDA.

Now with regard to precipitates, there are interim measures in effect. We recognize that firms may still be cited for this by an investigator. However, as long as the firm is working toward ways to resolve this issue and have procedures in place to not allow product with precipitates to get out to the consumer, then we would not take further action at this time.

Moving on to recalls: Recalls, some people don't understand, are really voluntary. Manufacturers choose to do this themselves based on potential for harm to the public. FDA mandated recalls are very rare. I can't remember us having done one. This would be an imminent danger to health situation. So what I am going to present are the numbers that the manufacturers themselves have done voluntary recalls on.

In allergenics the main issue, at least for this year, has ben sterility assurance. That is, the manufacturer is unsure about the sterility of the product on the market, and as a prudent measure has decided to recall that product.

As you can see again, there was some increase from '98 to 2000. Thus far this year, we

have nine on record. Again, one recall may involve several lots or several different extracts. So each extract was treated as one.

What are the most frequent issues that result in these enforcement actions that I mentioned earlier? Well, these aren't unique to this industry, I must say. This is pretty much commonplace throughout industry.

Probably the main issue is inadequate investigations when failures occur and what we call CAPA, which stands for corrective and preventive action taken to prevent those recurrences. These can entail failures during production and testing, handling of complaints, and again the precipitates.

In this industry we were seeing a lot of refiltration, reprocessing, and reworking. I won't define those terms unless you would like me to. I think refiltration is pretty self-evident.

There isn't anything that precludes manufacturers from doing these things, but if they do them, there has to be validation to support their use, and when there is a final product sterility failure, we do not feel that refiltration and revialing of the product is in the best interest of the public. So we are discouraging that in all cases.

The container/closure issues: These are the actual vial and stoppers that are used for the product. These need to be shown to be integral when they are in place over the life span of the product, and they need to be processed in such a way to guaranty that they are sterile and pyrogen free. These were some issues that were raised in earlier years.

Validation: The gentleman mentioned lyophilization. That is certainly one, but all the processes, the systems, the assays, as well as the equipment used to manufacture the products. There are some specific CFR regulations that were not being followed. We have a requirement for retention samples to be kept and inspected on a yearly basis to see that the product is still okay.

Again, the biological product deviation reporting: This is no longer a real issue, with one exception that I'll get to in a moment.

SOPs are standard operating procedures. It's an expectation that for everything a manufacturer does, they will have procedures in place to follow. We found that there weren't procedures in place for all processes or all testing.

Finally, we require an annual review of

each product to look at trends, see if there are problems that are starting to crop up, and this again is a CFR requirement.

Now where have we seen improvement? I like to report positive where I can. Certainly, with the container closure issue, manufacturers took that very seriously and we're no longer seeing a problem in that area. SOPs are in place. Some still need a little tweaking. They are there quite yet, but they are in place and being followed.

Validation is being addressed. Of course, that can be a long term action. It's not something that you can do in a day or a week or even a month at times. Refiltration, where being done, has been validated. Annual reviews and retention samples, those are being addressed.

Finally, the biological product deviation reporting has improved dramatically, as can be seen by the numbers, with the exception of the areas not related to precipitates. So if there is a stability failure, for example, that needs to be reported, and it's not -- we're not quite there yet.

Looking at kind of the future, the precipitate issue, we are very aware of it now in the Office of Compliance, and we've made the investigators

aware of it.

We are hoping to continue work with the manufacturers, and I'm sure the Office of Vaccines is really the most involved in this, but we are trying to keep in the loop so we know what progress is being made. We have made the BPDs allowance so that they can be reported en masse to, hopefully, help the industry as well as ourselves.

One issue I'd like to mention: We have a sterility test requirement under 610.12 of our regs, and it's actually a sterility method that is derived from the USP-22, United States Pharmacopoeia. We recognize that this is a very outdated regulation. We are currently in USP-24. All drug products -- that is, those regulated by CDER, Center for Drugs -- are required to follow the USP.

The CFR requirement allows for automatic retest when there is a failure and actually a repeat retest. In addition to that, there is no need for investigation into these failures to find out why they may have occurred. This is not in keeping with current USP.

So we are now presently discussing what to do about the situation, whether we are going to revise or eliminate 610.12(b). I think probably in the best

interest of everyone, it would be best to eliminate the reg and refer to USP. USP is always changing. There are revisions, updates all the time. As many of us know, regulations aren't quite that easy to change.

Before any decision is made, a policy statement will certainly be disseminated to the public, and we hope to move on this later this year.

So finally, just some contact information: If you would like to get some more information, the CBER home page is, of course, a wealth of that, not just in my area but in all areas affecting the biological products.

The BPD final rule that I mentioned earlier is available on the Web, and if you have any questions after today regarding compliance issues, please don't hesitate to call us in the Division of Case Management.

So I've tried to really condense this to try to keep everybody on somewhat of a schedule. So if you have any questions, I'd be happy to answer them.

DR. SOTO-AGUILAR: About the containers, are the stoppers latex-free?

MS. MALARKEY: I'm sorry?

DR. SOTO-AGUILAR: About the containers,

the vials, are the stoppers latex-free?

MS. MALARKEY: Latex-free --

DR. SOTO-AGUILAR: Is there any regulation about it?

MS. MALARKEY: We are currently working on that. I can't say that all of our closures are presently latex-free. Studies have to be done. I don't know in this industry. I'm talking in general terms, but it's a very big undertaking to replace the closures with latex-free.

Stability work has to be done. Integrity studies have to be done, and we are moving in that direction. I don't know if anyone from OBRR has any sense for the allergenics industry where we are.

MS. BRIDGEWATER: Yes. Some of the manufacturers are moving in that direction to change to latex-free stoppers. I don't recall exactly how many, which ones might be done, but it is a very time consuming process.

DR. UMETSU: With regard to the precipitate issues in the extracts, is that a new issue or it's just that it hasn't been reported in the past?

MS. MALARKEY: It hasn't -- My impression is it hasn't been reported in the past, but this has

been an issue that has been going on or has been identified for sometime. I think it probably came more to light during inspections, seeing it, you know, actually seeing it. So this now in the last couple of years has really become a very large issue.

CHAIRMAN OWNBY: Okay. Why don't we go ahead and take a 15 minute break. So we'll reconvene at 10:45.

(Whereupon, the foregoing matter went off the record at 10:28 a.m. and went back on the record at 10:46 a.m.)

CHAIRMAN OWNBY: Are there questions right now? Otherwise, we will move on tot he discussion of TSE and the questions of whether we need to be concerned about this with the allergenic extracts. Dr. Slater, I believe, is going to start that.

DR. FREAS: While he is getting set up, those of you who have been complaining that it's been cold, we apologize for that. The thermostat is set all the way up. Apparently, they are doing work down below. They have told us the heat will be on shortly, but it will take a little while to warm up.

CHAIRMAN OWNBY: You mean spring will come eventually.

DR. SLATER: We are now going to talk

about a topic that we have not discussed with reference to allergens before. That is the transmissible spongiform encephalopathies and allergen vaccines. I'm about to embark on a fairly lengthy excursion about TSEs before I get back to allergens.

So what I'd like to do before I do that is give my summary in advance, so you know what direction we are heading in, in terms of the allergenic products themselves.

In summary, most allergen extracts are produced without any bovine components other than glycerol. Glycerol, just again to give you a punchline in advance, is not a problem in terms of TSE for a number of reasons. However, among allergen extracts, mold extracts are stored and propagated in culture media, some of which contain bovine components, and some of these are of uncertain origin. However, the risks associated with these contaminations are minimal, and I will show you these calculations as well.

So what are the transmissible spongiform encephalopathies? Well, it's a group of fatal neurodegenerative diseases that are transmissible. They are known to be transmitted from one organism to the other.

They are encephalopathies. They are conditions characterized by abnormal brain function, and the spongiform refers to the microscopic appearance of the brain tissue in affected patients with the formation of holes or vacuoles and scarring or gliosis. So the name itself includes both evidence about the epidemiology of the disease, the clinical appearance of the disease, and the pathological appearance of the disease.

Well, what's the clinical appearance of the disease? It's somewhat variable, but it almost invariably contains some cerebral symptoms, including dementia, behavior changes, visual disturbances. There are often cerebellar symptoms, ataxia, nystagmus and speech abnormalities; pyramidal signs and symptoms, spasticity, weakness, and hyperreflexia; extrapyramidal signs and symptoms such as tremor, myoclonus, rigidity and akinesia; and invariably, death. These are degenerative, fatal diseases.

Pathologically, they are characterized by gliosis or proliferation of glial cells, neuronal loss, vacuoles, and amyloid plaques, and the amyloid plaques are variable. They are not all associated with -- They are not all present uniformly in all of the diseases. They are variable in CJD, but they are

universal, for instance, in the new variant CJD.

Now the first transmissible encephalopathies were described in animals, and of course, they weren't always known to be transmissible spongiform encephalopathies. These were known in the past as the sort of sloe viral diseases. When I was in medical school, that's how we studied them.

Scrapie was the first to be described. It's a fatal, progressive disease of sheep, and it was described in 1932 -- I'm sorry, in 1732. Scrapie affects sheep and goats naturally and can be transmitted experimentally to several other animal species.

Now chronic wasting disease is a rare disease of elk and deer, primarily in the United States. It happens both in captive populations and in wild populations, and it appears to be a disease that is pathologically very similar to the other TSEs that have been described.

Transmissible mink encephalopathy takes the form of a rapidly developing epidemic, usually involving single mink farms. Again, the disease is uniformly fatal.

The bovine spongiform encephalopathy is the one that, of course, concerns us most. It was

recognized as a potential member of the scrapie family as soon as it was first discovered in 1986 and shows all the pathological characteristics of TSE.

Now feline spongiform encephalopathy, which appears in cats, is clearly a derivative disease of the bovine encephalopathies. In other words, clearly, it is transmitted to cats who are fed contaminated materials from cows, and the epidemiology follows fairly precisely the epidemiology of the bovine encephalopathies.

Of the human TSEs, the first described was kuru, which was described in the 1950s in isolated tribes in New Guinea. It was spread by the ingestion of brain tissue of deceased relatives, and the prevalence of this disease decreased rapidly after the cause of it was identified and the practice discouraged.

Kreutzfeldt-Jakob disease, CJD, occurs in about one case per million individuals per year. This is the sporadic form of CJD. It also appears in a familial form and in an iatrogenic form, by the transplantation of neural tissue contaminated with CJD, and in addition there is a new variant form that is associated with transmission of bovine TSEs.

Now these other human TSEs are much rarer,

and I am not going to discuss them, but simply note that there have been other human TSEs described. But there is a key point that has to do with the pathogenesis of these diseases, that the diseases occur both in what appears to be endogenous cases -- in other words, the familial or genetic cases of human TSEs -- and what appear to exogenous cases, the clearly infectious ones.

What's important to note is that, whether you have a so called endogenous type or an exogenous type, your tissue is infectious. So that's a key point to remember when we get to talking about the pathogenesis of the disease.

This is a slide to show the epidemiology of bovine TSE, and the point of the slide is to show that bovine TSE was isolated in both time and place. The epidemic of bovine TSE was fairly well defined. It started in 1986, and it peaked in around 1991-92, and it has been dropping rapidly since that time.

So the interval between 1986 and, certainly, 1995 to 1997 were certainly the peak periods. It hasn't dropped off to zero quite yet, but it has been dropping quite rapidly and, clearly, this is a well identified interval in time in which bovine TSE seemed to be occurring quite a great deal.

Now this is a little harder to read, and these are the different countries in Europe and the number of cases of bovine spongiform encephalopathy detected. This is cumulative detected through the year 2000, and you can see here that the epidemic is localized in terms of place as well, nearly 180,000 cases in Britain. The next nearest country is Ireland which had 540 cases, Portugal 473, and on down from there. So the epidemic is localized in terms of time and relatively localized in terms of geography, although as you can see, there have been cases reported in Europe as well.

What you have here in the lower righthand corner are the cases of new variant CJD, which appeared in '95, '96, and '97, all at that time in the United Kingdom.

Now what about this new variant CJD? The new variant CJD can be distinguished on clinical grounds from the classic CJD, on the basis of the differences that are demonstrated here.

The time course of the new variant from diagnosis to death is somewhat slower, from eight months to 16 months. The EEG changes that are very common in the sporadic form of CJD don't appear in the new variant CJD.

The clinical presentation tends to be more behavioral for a longer period of time in the new variant than it is in the sporadic form, and the age at presentation is significantly younger. The sporadic, the mean age is 60, whereas the new variant has a mean age of 28 with a range of 16 to 52.

Note here that the incidence of the sporadic is, as I said, one case per million per year. Even at its peak in the United Kingdom, there were 22 cases over a period of 1994 to 1997 or for an incidence of 1.5 times 10^{-7} per year.

So even at the peak of the new variant CJD epidemic, if you had CJD and weren't clinically distinguished between the two, you had more of a chance of having the sporadic than the new variant form. But, clearly, this was an epidemic, because the new variant doesn't occur elsewhere.

So what are the pathogenesis clues? CJD occurs in both familial and infectious forms. The infectious material is insensitive to UV and ionizing radiation, has a low ratio of nucleic acid to protein, is partially susceptible to treatments that are known to destroy proteins.

Hence, in 1982 Dr. Pruzner formulated the word prion for proteinaceous infectious particles that

he proposed to be the infectious particle associated with the spongiform encephalopathies. This is a group of 33 to 35 kilo-Dalton sialoglycoproteins.

It's important to note that these are normal proteins. They are present in CNS tissue. they are present in non-CNS tissue, and there is a normal cellular isoform so called PrP^{c}. However, the prion proteins appear to be transmissible agents for TSEs.

The conversion, the infection with a TSE is associated with the appearance of protease resistant prions, which are designed either PrP^{res} for protease resistant, or PrP^{sc}, short for scrapie.

Infection with PrP^{res} or PrP^{sc} induces conformational changes in the normal cellular isoforms that are associated with a switch from an alpha helical formulation to a beta pleated confirmation, and associated with increased insolubility and aggregation of the proteins.

Now susceptibility is a function of the primary sequence of the PrPs that are involved. There is a species barrier associated with infection with TSE. That appears to be a function of the differences between the PrP sequences among different species.

There even are intraspecies polymorphisms

which may in part account for differences in susceptibility of individual organisms. And some of these susceptibility factors can be overcome with a transgene. In other words, the experiment is, if you try to infect a mouse with a bovine TSE, you can overcome the species barrier by giving the mouse a transgene with bovine normal prions, and you can especially do so if you ablate the original murine PrP gene.

Now this doesn't account for all infectivity factors. So you still can be infected. Knock-out mice, for instance, that have no prion protein at all have quite a significant barrier to infection.

This is simply a three-dimensional model of the mouse and hamster PrPc. You can see the alpha-helical confirmations, and what you can see is that there's -- in the normal confirmation there is a minimal beta-pleated sheet in this region, but this expands when the confirmation is changed by the appearance and aggregation with a mutant PrP.

Well, how do you get from a inoculation to disease? Typically, inoculation is by GI absorption. The abnormal prion proteins replicate, and they replicate in the sense of inducing conformational