FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

ALLERGENIC PRODUCTS ADVISORY COMMITTEE

(Open Session)

Monday,
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Versailles Ballroom I
Holiday Inn Bethesda
8120 Wisconsin Avenue
Bethesda, Maryland

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C O N T E N T S

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P R O C E E D I N G S (8:00 a.m.)

DR. FREAS: Good morning. I'm Bill Freas. I'm the executive secretary for this advisory committee. I would like to welcome everybody here to the 15th meeting of this Allergenic Products Advisory Committee.

Most of our advisory committee meetings for this committee are by teleconference. This is our first face-to-face meeting since November, 1994, and I would just like to welcome all the members who are not used to coming to Bethesda for their travel down here. Believe me, they do come from long distances.

This morning's session will consist of presentations and committee discussions that are open to the public. Later this afternoon, we will hold a short closed session until the meeting is adjourned, as described in the Federal Register notice of February 9th, 1999.

At this time, I would like to introduce to the audience the members sitting at the head table. If the members will raise their hands so the audience can identify them, I will start on the right-hand side of the room. That's the audience's right-hand side.

Our first member is Dr. Te Piao King, associate professor, Rockefeller University.

Coming around the corner of the table is Dr. Andrew Saxon, professor of medicine, Division of Clinical

Immunology and Allergy, UCLA School of Medicine.

Next, I would like to introduce to you our chairman of this committee, Dr. Dennis Ownby, professor of pediatrics and medicine, Medical College of Georgia.

At the next seat is our newest committee member, and I would like to welcome Dr. Dale Umetsu, chief, Division of Allergy and Clinical Immunology, Department of Pediatrics, Stanford University.

Around the corner of the table is Dr. Daniel Ein, clinical professor of medicine and allergy, George Washington University.

Next is Dr. Betty Wray, professor of pediatrics and medicine, Medical College of Georgia.

Also sitting at the table for this morning to aid in the presentation, but not part of the committee, is Dr. Thomas Hoffman, acting director, Division of Allergenic Products and Parasitology.

I would now like to read into the public record the conflict of interest statement for this meeting. "The following is made part of the public record to preclude even the appearance of a conflict of interest at this meeting. Based on the agenda made available and all relevant data reported by participating members, it has been determined that all financial interests in firms regulated by the Center for Biologics Evaluation and

Research that may be affected by the committee discussions have been reviewed.

"To avoid even the appearance of a conflict of interest, Dr. Ein has recused himself from the general discussion on the proposed package insert for allergen extracts later today. Dr. Ein is permitted to participate fully in other committee discussions and deliberations.

"No waivers were necessary for this meeting under Section 208.

"In the event that 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 such discussion, and their exclusion will be so noted on the record.

"With respect to all other meeting participants, we ask in the interest of fairness that they address any current or previous financial involvements with any firms whose products they may wish to comment upon."

I would also like to announce that three of our committee members that would have liked to have joined us are not here with us this morning. They are Dr. Henry Claman from the University of Colorado Health Sciences Center; Dr. Gail Shapiro from the Northwest Allergy Center, and Dr. Shapiro will be joining us later on in the

afternoon by teleconference; and also our consumer representative, Nancy Sander, from Mothers of Asthmatics, Inc. will not be joining us this morning.

Dr. Ownby, I'd like to turn the microphone over to you.

DR. OWNBY: Thank you.

I'd like to welcome everyone here, especially members of the audience, the general public, those members representing the FDA, and especially the committee members. It seems a little strange actually to sit here in the same room with everyone after a number of teleconferences and some of the attempts at the videoconferences and other things that we've had that haven't always worked quite as well as the technology is advertised.

I hope that this can be a relatively informal session. I would like to encourage as much discussion as is relevant to all of the issues that will come up, and I hope this can be very productive for everyone. It does, I'm afraid, fall to the committee chair to try and make sure that we stay on the agenda and complete the tasks assigned to us today, but I'm confident that that won't be an onerous problem with this group.

I believe our first speaker is Dr. David Feigal, deputy director for medicine in the Center for Biologics Evaluation and Research.

DR. FEIGAL: Good morning. I wanted to come this morning and add my welcome and make a few comments on what are the recent, short-term, and future prospects for CBER's work in this area. I particularly enjoy coming to advisory committees because actually that was one of my first introductions to FDA, serving on an Antiviral Drug Advisory Committee from 1989 until 1992.

This has been a period of time that has been a time of shrinking resources for CBER and this has been one of the areas that has not been protected. The kinds of protections that occurred began in 1992 with the passage of the Prescription Drug User Fee Act, which created user fees for certain types of applications, but excluded other areas. One of the upshots of this has been that when we have had reductions in our budget, the user fee specified areas were protected and the unprotected areas had to then absorb the entire budget cuts for the center.

The cuts actually, on the face of them, didn't sound very large. They typically were on the order of a 2 percent cut. That would actually be multiplied by cost of living and salaries that had increased by another 4 percent. But when this goes on after four or five or six years, you realize that that 4 percent is now 25 percent, and it has to be absorbed by a small part of the agency.

One of the things that I think has happened --

and I would encourage you, as advisory committee members, to be part of the process that comments on this, because you are some of the people in the outside world, not in industry, not in that part of the customers, if you will, that we serve that can comment on the important parts of our mission -- there has been continued public attention to the safety and the quality of the products approved by the agency. We are seeing proposed for the first time in five or six years actual increases in the budget that have made it through the administration, made it through the Office of Management and Budget, that would actually increase the base in the next year's budget for review in the non-user fee areas, and I think this is a very important, very significant movement back and away from that.

In one way, these aren't the concerns of this committee, but it has impact in terms of how well the agency is staffed and funded to operate in these areas, in terms of the level of the quality and detail of the materials that we can bring to you and the timeliness that we do it, so it indirectly is your business.

I'd like to thank you again, along with Bill, for coming and for meeting in a public forum. As you know, these meetings are much more widely followed than the size of the audience that attends, because the transcripts are publicly available and widely watched not just by industry,

but consumer groups. So again, let me extend my thanks for your coming, and I look forward to a productive session.

DR. OWNBY: Thank you very much.

I believe our next presentation will be by Dr. Thomas Hoffman, who is the acting director of the Division of Allergenic Products and Parasitology.

DR. HOFFMAN: Thank you, Dr. Ownby, ladies and gentlemen.

It's my pleasure today to introduce and focus the session somewhat, to give you a sense of some of the things that we'll be talking about. This is basically in the form of an update for the committee, in part due to the reasons that have been mentioned, the long time between face-to-face meetings, but there is also a background of a number of issues that are going on that have raised questions about our ability to adequately perform the task that's set before us. So in order to have a basis for discussing that, I'd like to give you some sense of understanding of what the task is.

Currently, the Division of Allergenic Products and Parasitology is organized into four units, of which the Laboratory of Immunobiochemistry is one. Virtually all of the discussion today will focus on the Laboratory of Immunobiochemistry, but you will clearly see contributions of other members of the division, other units of CBER, such

as the Division of Policy and the Office of Vaccines, and general contributions based on the wide range of expertise that exists.

The three other laboratories, the Laboratories of Biophysics, Parasitic Biology and Biochemistry, and Immunoregulation, perform research that's related to either immunology, measurement, computer modeling, analytical methods, or general cell biology.

The mission statement of our division is outlined in the FDA staff manual guide and, as you can see, one of the major focuses of our efforts is to do research that relates to allergenic and some of the other products in the division. I hope you'll see evidence that we are hard at work on this aspect of our job, that the laboratory evaluation of allergenics and the regulatory procedures that we use to review products that come before us, both in terms of manufactured products and investigational products, our laboratory and research programs support these intensively, as I think will become very evident.

We have, fortunately, a number of very capable clinical individuals who provide the expertise necessary for the clinical aspects of the review, and we're very fortunate to have excellent collaboration with our colleagues in the policy office and in the Office of Compliance, as you will see today, to help regulate all

aspects of the manufacturing and clinical processes related to the approval of allergenic products.

We also serve as a resource, a body of expertise, that is called upon by various parts of the Public Health Service and the federal government for other issues related to allergy in general. These would include drugs for allergy, international policies that are being developed for harmonization, and trying to standardize allergenics around the world.

We are not, fortunately, starting from scratch here. We have a long history of work in this area. It goes back into the '50s and early '60s. We are well aware of the daunting task of regulating allergenics. A wide variety of skills, attributes, and knowledge would be necessary to competently regulate allergenics. These obviously begin with a basic knowledge of clinical immunology and allergy, and knowledge of the regulations, which sometimes seems very easy, but sometimes can be quite abstruse.

The clinical program, as you will hear today, has contributed immensely to the current status of allergenic regulation in the United States and around the world. More and more these days, analytic issues are coming to the fore. These pertain to characterization of allergenics and, with the advent of more molecular

biological techniques, an understanding of each and every component of a potential allergenic product.

We're having to give more and more attention to some of the quality control or quality analysis issues as the number of products come into the realm of standardization and as we consider standardizing new products. This requires a fairly intense coordinating effort, since not everything can be done in our division, and requires a good deal of management expertise and, as I think we are participating in today, a public relations effort to make people understand what we do and to have us be responsive to the needs of our community.

In the division, I know everyone, including the center director and the office director, shares our commitment to research. We are very proud of our research accomplishments, and we see this as the fundamental basis for understanding what we're going to do in the future and how we're going to do it.

That's the end of my slides. I will come back at the end of some of the open session to focus some of the deliberation, but I welcome you to ask any questions of myself or any of the people that present. We have Dr. Egan here as the acting director of the Office of Vaccines. I'm sure he'd be able to respond to any global or policy questions. We are here to have appropriate discourse and

to have you know us better, and maybe get to know you a little bit better as well.

DR. OWNBY: Are there any questions from the committee for Dr. Hoffman?

DR. SAXON: This is Dr. Saxon. I understand the first three laboratories' mission reasonably well. I'm not quite clear what the Laboratory of Immunoregulation does. Without going into great detail, could you just give us an overview quickly of what's involved?

DR. HOFFMAN: The Laboratory of Immunoregulation does fundamental research in immunology. They are focused on issues related to HIV infection, the immune response to HIV, and mechanisms for HIV entry. They are a cadre of -- cadre. There are two of them, clinical immunologists, who also participate very intensively in the review of allergenics. Dr. Berkower will be here later, Ira Berkower, and Carol Weiss.

DR. SAXON: Thank you.

DR. OWNBY: Any other questions from the committee members? Dr. Hoffman, I did have a question. We've heard of some of the budget restraints you've had and the idea of reorganization. Would you care to comment on how that is going and where your plans are at the present time?

DR. HOFFMAN: Sure. We acknowledge the fact

that it's necessary to manage the resources in somewhat different fashion, given some issues pertaining to scarcity. We have under consideration a proposal in the Office of Vaccines to undertake some organizational shifts, which would basically involve in toto movements of laboratories of our division to other divisions within the Office of Vaccines. There is no contemplation of moving any of the resources, individuals, or programs outside of the Office of Vaccines at this time.

In my view, it's simply a control shift that involves management supervisory authorities, but I think you'll see today that the basic program in allergenics is going to remain intact. If anything, there's a commitment -- we have this clearly from Dr. Egan and from Dr. Zoon, the center director -- to augment the resources of the allergenics program, both in terms of personnel and in terms of funds.

DR. OWNBY: Any other questions or comments?

(No response.)

DR. OWNBY: Thank you.

I believe our next speaker is going to be Dr. Jay Slater, who's chief of the Laboratory of Immunobiochemistry.

Jay?

DR. SLATER: Thank you very much, Dr. Ownby.

It's a real pleasure to be here. Let me just introduce myself. I've just joined the FDA six months ago, and I came here from 12 years at Children's Hospital. I am the head of the Laboratory of Immunobiochemistry.

My talk today is going to focus on three aspects of our activities. The first period, we'll be talking about operational issues at the lab. The second period, we'll be talking about some of the research efforts that have gone on in the past and my feelings about what directions we should go in the future. In the third part, we'll talk about some specific regulatory activities of the lab that we're considering modifying over the course of the next several months.

Let me just orient you a bit in terms of the materials that you have. The materials that you received today, the top portion represents the hard copy of all the slides I'm going to be presenting over the next two and a half hours, so that may be something that you will want to refer to. That's in the heavy clip.

Immediately behind that is a list of abbreviations that may seem fairly redundant and elementary, but I wanted to make sure that everybody was on target in terms of what I was talking about and that I didn't unnecessarily lose anybody.

The missions of the Laboratory of

Immunobiochemistry are, as I see it, multifold. I think research is a major part of our mission, but of course we are concerned very much with product quality. I think, as you'll see, especially in the research portion, I've made a major effort to construct a research program in which each individual item is closely related to issues relating to our regulatory activities of these products. We certainly consider ourselves a support unit for the other regulators at FDA, and we consider ourselves a support unit for people in the manufacturing community.

The stewardship of the Laboratory of Immunobiochemistry was in flux, and in fact one of the major problems that I hope to solve early and efficiently over the next year or so are the problems associated with rapid turnover of personnel. Yuan Lin was my predecessor, and she left LIB in the summer of 1997. During the hiatus between her departure and my arrival, two people from the division, Paul Turkeltaub and Rich Pastor, alternately were acting chiefs of the lab. This was a largely thankless job for both of them. They put in a lot of hard work trying to get the lab on track. They did really a very good job, and in fact I really want to thank both of them, because when I arrived at the end of August, many things were really running very smoothly, and I had a good honeymoon period in which I had very few things to worry about.

Rich Pastor, in particular, has been a continued help to me in terms of understanding the operations of the laboratory, understanding some of the theoretical underpinnings of some of the work that we do, and a lot of his thinking and a lot of his hard work since I arrived has gone into much of the material that you'll see about an hour and a half from now, and we'll talk about that later.

The staffing of LIB at this point, full-time, me, and Lyudmila Soldatova is a Ph.D. She's our postdoc in the laboratory. She's doing very important work involving bee venom proteins and is also helping out with a number of other studies that we're doing on the characterization of allergens, which we'll be talking more about later.

Maneesha Solanki has been in the lab for several years. She's one of the research associates. She is very experienced and certainly has been a great help in terms of keeping the lab running, especially during periods of instability, and also, after my arrival she has really been very, very helpful.

Beth Paupore was my research associate at Children's since April, 1997, and I was very fortunate that she agreed to come to FDA this fall when I came here.

In addition, I want to thank Al Gam, who is a biologist in the parasitology laboratory. Again, during

the hiatus in which we were relatively understaffed, he helped out a great deal, and has, at my request, really continued to be involved in the laboratory activities, again giving us a lot of help with his assistance and experience.

Gerry Poley is a guest worker from Children's Hospital and Li-Shan Hsieh is a Ph.D. who used to work in the lab and now works with us part-time, although she has moved over to CDER.

The routine regulatory activities of the lab include protocol review -- that is, review of protocols that are sent in to us by manufacturers on their analyses of particular lots -- testing of the products, reference development, reference distribution, and reference maintenance, including semiannual checks and replacement of references as they become out of date or old.

One of the things that had been started before I arrived was the process of optimizing one of the important assays that the laboratory does on a routine basis, and that is the competitive ELISA for relative potency.

I wanted to call the attention of the committee members to the package that you were sent before coming here. The next to the last item is a memo describing the proposed changes in the competitive ELISA that was sent out

to the manufacturers.

The purpose of this reevaluation was really to critically reevaluate the method, make sure it was working as best as possible, and then finally to validate any changes that we made. In fact, the results were very reassuring, and that was that almost all the changes that we made were merely refinements of the conditions that already were in our competitive ELISA protocol. Those things that weren't refinements were truly minor changes.

For instance, we changed the buffer detergent from Brij to Tween-20. That seemed to work somewhat better. We used the same blocking buffer and diluent buffer for all the different preparations using 1 percent BSA. Again, no radical changes there, but just a standardization of things that have been not entirely clear in the previous protocol.

The coating, composition, and conjugate incubations were converted to absolutely overnight, as opposed to four hours to overnight, and the substrate that we used we specified that it should be equilibrated to room temperature for five minutes and the incubation steps should be exactly for five minutes. Again, in the previous protocol, these things were left not specified.

When we looked at the revised protocol and looked at three common allergens that we typically

evaluate, the results in fact were extremely reassuring, and that was that for each of these we tested extracts that were at a relative potency of 1. In other words, they were where we wanted them to be. We also tested them at double that relative potency and at half the relative potency, and fortunately, none of the 0.5 or 2.0 extracts passed, which is reassuring. None of the 1.0 extracts failed -- also reassuring. In fact, the standard deviation of our evaluation was for the most part well within and significantly smaller than the old standard deviation limits, which were 0.1375.

So we found that, for both theoretical reasons and for practical reasons, this was an improvement over the previous regimen. We sent out a memo to the manufacturers advising them of these proposed changes and we're waiting for feedback from the manufacturers regarding that.

In addition to that activity, some of the more routine activities that have continued have included reference replacement activities. For instance, we replaced the cat S2 sera by cat S2a, mite S3 by S4, latex S2 sera was replaced by another pool, S3, and I'll have some more information about that in the next few slides. Both the D. pteronyssinus and cat extract replacement activities are in progress, and we'll be talking more about reference replacement in just a few minutes.

As I said, one of our specific projects that I was involved in fairly soon after coming was the replacement of the latex serum pool. In fact, after much evaluation of the various sera and plasmas that we had available, we formulated a pool from seven adults with latex allergy. This was actually just prepared a little over two months ago. We were happy that all of the bands in our standard latex antiserum E8 was detected, and in fact the relative potency data using that extract was very reassuring as well.

What you see here is the absorbance plotted against the log dilution of E8, which is our standard latex extract. This is the absorbance with PBS, which reassuringly is at zero. The two E8 runs were exactly overlapping. Again, that's fairly reassuring.

This is another product that we had in our lab that's actually at a concentration of 1 milligram per mL, another latex extract, and again, reassuringly, this is obviously a perfectly parallel line to the other run that we had done with the standard E8.

DR. SAXON: Jay, I'm confused for a minute.

DR. SLATER: Sure.

DR. SAXON: Are you talking about serum or antigen?

DR. SLATER: We are testing a new antiserum

with the antigens that we have in, so we want to see whether the numbers that we get are what we would expect to get.

DR. SAXON: I was just getting confused between E8, which is an antigen. E8's an antigen.

DR. SLATER: Absolutely. No, that's right. E8 is an antigen, and we're simply testing the new latex sera pool.

DR. SAXON: So this is one sera. You're not comparing your sera to anything else. You're just looking to see --

DR. SLATER: That's right, but these are the results that we expected based on our data from S2.

DR. SAXON: I understand.

DR. SLATER: And getting more precise, it turns out that E8 is a fairly concentrated standard, 3.9 milligrams per mL. The unknown that we were using was 1 milligram per mL. The relative potency of E8 was 95 percent confidence between 0.89 and 1.35, and X, the confidence interval was 0.19 to 0.30. Again, really pretty much what you would expect based on simply the protein content of the products. That was reassuring about this new latex serum pool, and we have a lot of this, so we're confident that we're going to be moving forward with more latex work, but again, I'll refer to that a little bit more

later.

One of the other operational issues that this lab began to address over a year before my arrival in the summer of 1997 was the issue of mite stability. The issue of mite stability is of particular operational importance to a laboratory like ours, and the reasons that the lab began to investigate this were that it has been known for quite awhile that there are cysteine and serine proteases in mite antigens, there have been conflicting prior data on stability -- in particular, Hal Nelson's paper in '96 and Yuan Lin's paper in '98 came up with some ambiguous information about the stability of mite antigens -- and we obviously are very concerned with the issue of possible short shelf lives of reference materials.

In particular, it was called to our attention by some manufacturers that they were concerned that our reference materials were not stable, by virtue of their finding that when we switched reference materials there were abrupt changes in the relative potencies of their materials. This is something that obviously was of great concern to the manufacturers, and it was of concern to us as well.

So in 1996, Yuan Lin began a series of studies using mite extracts that was actually published in the Annals of Allergy, Asthma, and Immunology in 1998, and that

is one of the reprints that is in your handout that you got before coming here. I'm going to show some of the slides from that study.

The study looked at various mite extracts stored 4 degrees, 26 degrees, 37 degrees, and, believe it or not, 50 degrees for between six and 30 months, although the 50 degree samples got dry pretty fast, and so they obviously didn't have much to look at after six months.

What they found, using a relative potency at 4 degrees as their standard, as their benchmark, they found actually relatively little deterioration of relative potency over six to 30 months at room temperature, which was actually remarkably reassuring. At 37 degrees, there was some dropoff, and at 50 degrees, obviously there was a very rapid dropoff. Unfortunately, as you can see, there's a design question with this experiment, and this actually was addressed subsequently by other members of the lab before my arrival.

Again, going back to the paper from 1998, in spite of the stability of the relative potency preparations, there was significant instability of the specific mite allergens as measured by monoclonal antibody-based ELISA assays, and this is a panel of Der p 1, and up here on the top curve is the 4 degrees, 26 degrees, and 37 degrees, and the 50 degrees is right along the baseline

here for Der p 1. For Der p 2, again, 4 degrees, 26 degrees, 37, and 50 degrees, and the abscissa here, I admit, is fairly hard to see, but the first time points are three months, six months, and nine months.

Looking at the Der f antigens, Der f 1 appeared to be relatively stable over the period of observation, but Der f 2 really had a very similar pattern to the other allergens that were looked at.

So there was actually a contradiction in this paper, but the contradiction was a little hard to sort out, because the standards and the benchmarks that were being used in the two studies were rather different. They found that Der p 1, Der p 2, and Der f 2 were unstable at 4 degrees, Der f 1 was stable at 4 degrees and unstable at greater than 26 degrees, but the RP was conserved at 26 relative to 4, but they didn't look at what the RP did at 4 relative to -20 or relative to a lyophilized preparation. So that was the first thing that my predecessors wanted to look at in a more careful way. In addition, they wanted to look at the possibility that protease inhibitors might somehow retard this process.

The objective of the study was to look more carefully at this question to identify and characterize possible degradation in glycerinated mite extracts with or without inhibitors, to store this time at -70, -20, 4

degrees, and 37 degrees for six to 12 months, compare them this time to a lyophilized standard, which was provided for us by one of the manufacturers, and then to look at this by three assays -- the competitive ELISA for relative potency, the two-site ELISA for specific Group 1 and Group 2 antigens, and Western blot using both antisera and monoclonals. Now, I have only some of the data to show you, but I do have some material that I'd like to take you through.

Again, let's look at the relative potencies. Unfortunately, this is a complicated slide because you have four different mite extracts, you have the six-month data and the 12-month data, and you have either three or four bars at each segment. That's because at the six-month point we don't have the assay results for the -70 products, but at the 12-month point we have the assay results for all the different products.

I just want to focus your attention at the maroon bar and at the beige bar to the right of it. The maroon bar is the -20, the beige bar is the 4 degrees, and what you see is actually a very reassuring stability of relative potency of the 4 degree product compared to the product stored at -20. Again, 4 degrees, -20, 4, and -20. The error bar on this is a little bit impressive, isn't it? And remember, the benchmark here in this assay is an RP of

1, and our standard is anything between .7 and 1.4.

So the results of the relative potency assay are fairly reassuring that not only from Yuan Lin's previous study do we know that 26 degrees is stable compared to 4, but now we can answer the more important question, and that is the stability of samples stored at 4 degrees compared to both -20 and compared to the lyophilized standard which was taken as an RP of 1.

Notice an interesting observation that with several of the preparations, the -70 one is significantly lower, and that actually makes good intuitive sense, because with 50 percent glycerol, the sample doesn't freeze at -20, but it does freeze at -70. We know that freeze/thawing cycles tend to decrease relative potency of stored extracts.

When we use the monoclonal antibody-based assay to look at Group 1 and Group 2 allergen content -- and these data are only from six months. We haven't completed our analysis of the 12-month data -- we actually find that there is some deterioration relative to the lyophilized preparation. In this one, the standard is a stored standard from the kit, which is actually stored at 4 degrees, but we see here that the lyophilized preparations in several of these have significantly higher allergen content than any of the others, but for some of them it

doesn't make that much of a difference, but what we notice is that, again, between the -20 and the 4 degrees, the maroon and the beige, there is no significant difference in allergen content.

So again, the information here is not yet complete. We are continuing our analyses. We actually did see on Western blots some loss of protein bands at 4 degrees, but it was not clear exactly which bands these were and the results weren't entirely consistent, but we are concerned that we may be losing some specific antigens at 4 degrees when defined by molecular weight on a Western blot. However, in general, our tentative conclusion so far is that relative potency is stable at 4 degrees relative to lyophilized, and that's an important question that we wanted to answer.

We do know that there is some loss of protein bands at 4 degrees, and we do know that there is some loss of specific mite allergens compared to lyophilized at 4 degrees, and in fact at -20, but this doesn't appear to correlate with the relative potency.

In data that I have not shown you, simply because I'm showing you enough data today, the protease inhibitors do not appear to offer any protection at all, at least not in the way that we use them. So we don't think that that's necessarily an answer in terms of preserving

what little mite activity was actually lost.

I'm going to switch gears a little bit to talk about our reference --

DR. SAXON: Can I ask you a question?

DR. SLATER: Absolutely.

DR. SAXON: I want to clarify, though, so I understand it. So since the relative potency doesn't change, yet you have a loss of some bands or antigens by monoclonals, what you're suggesting is basically you're just losing a couple of epitopes that may be -- one part may be degraded, but you're really not losing anything, and that showed maybe -- I won't say a flaw, but a difficulty with using a monoclonal antibody assay for these.

DR. SLATER: I couldn't have said it better. I think that the monoclonals may be detecting specific linear sequences that, even when eliminated, lead to no significant decrease in confirmational epitopes or even other linear epitopes that are recognized by the serum.

Now, remember, again, forgive me if I say obvious things, but the competitive ELISA is based on polyclonal human antisera that may represent half a dozen significant mite allergens, and therefore the loss of part of one of them may not be recognized within the significance limits that we have.

In fact, if we had a more precise assay, we

might actually see a real loss, that it might be a 5 percent loss or a 10 percent loss. With those error bars, you need to recognize that you wouldn't be able to recognize that kind of loss.

But thank you.

DR. OWNBY: Jay, apropos of that, has anyone done the work of absorbing out one of the major mite allergens to see what loss in potency you get acutely if you use one of these monoclonals to absorb all Der p 1 out, for example?

DR. SLATER: I'm not aware that that has been done.

DR. TURKELTAUB: Yes, it has.

DR. SLATER: I'm being corrected.

DR. TURKELTAUB: A paper by Albers, 1998. This is Paul Turkeltaub. They desorbed Der p 1 and looked at its effect on histamine release in skin test reactivity. It had no effect. Maybe 5 percent. It's not a major allergen. It's a minor --

DR. OWNBY: So that would explain what we're seeing in Jay's data. That would fit with what we're seeing in Jay's data, where you can lose one of these monoclonally-defined allergens and not affect the overall potency.

DR. TURKELTAUB: Yes. In my talk, when we

looked at what was the potency assay for mites in the mid-80s to the advisory committee, Der p 1 was proposed as a major allergen. We looked at RAST inhibition. That correlated with skin test relative potency. When we looked at Der p 1, that did not correlate with skin test relative potency. Der p 1 did not correlate with RAST inhibition relative potency. We knew that in 1986. Albers published in '88 when you desorb out Der p 1, there's no effect on the overall allergenic activity, except maybe 5 percent, which was well within the limits of the variability of the assay.

There are a number of other data which I'll discuss later on about why some these "major" allergens are not major allergens at all. It's a misnomer and results in misleading impressions in the allergy community about using such estimates.

Now, Jay hasn't mentioned anything at all about the quality control issues, about how replicable even Der p 1 estimates are. In Yuan Lin's paper, using reagents from the same supplier, she got three-fold discrepancies in the estimates. So lot-to-lot consistency of those reagents -- and I think Yatswada, a Japanese investigator, tried to estimate Der p 1 using another set of reagents, and comparing them among labs couldn't get comparable results. There may be some quality control issues as well, as well

as, and I don't know if Jay may want to comment, within-lab reproducibility, using the same sets of reagents and the same references, and whether you get the same reliable results.

I don't know if Jay wants to comment or not. I mean, he's had the hands on experience.

DR. SLATER: I'll tell you, I don't really want to comment on it, because I'm not sure it's broadly relevant. I think that certainly there are problems with any of these assays and, as those of you who have worked with monoclonals know, they each have a personality of their own in terms of how they can be handled and what they recognize.

Yes, I think that, again, the purpose of this study was not to impugn the general use of monoclonal-based assays, but clearly, in order to accomplish that, you really need to know what the important allergens are, what actually goes into it, and I think, Dennis, your question was right on point in terms of that.

MR. GAM: Al Gam. Just one other comment. Just because a band disappears on a Western because there's some breakdown of the allergen doesn't mean the epitope's not still there.

DR. SLATER: Right.

MR. GAM: And you may still see it in a

serologic test, whereas that band is gone in the Western.

DR. SLATER: Right. The band may just appear under a different band altogether on the blot.

MR. GAM: Exactly.

DR. SLATER: Thank you.

The reference replacement program. Committee members, in the handout you got today, behind the abbreviations, there is a four-page summary of our time table for reference replacements, and you may or may not wish to look at that now.

One of the things that we found shortly after my arrival was that many of the references that we had in stock were actually, formally speaking, out of date. Now, I just want to preface this by saying that LIB has had a fairly aggressive reference quality control program for several years, including blotting and competitive ELISAs done every six months to really monitor the appearance and the potency of these products. That being said, 20 out of 24 of the products that we looked at were formally out of date. So one of the things that I wanted to do was to bring our inventory up to date, and that's what that reference replacement program shows in your handout.

Again, the idea is to bring the full inventory up to date, with a target completion date of August, 2001. That seems awfully far in the future, but actually that's

fairly aggressive scheduling for us, because there are a lot of references and it involves not only activities by us, but the manufacturers as well. Getting a new extract into play is an iterative process between us and the manufacturers, so we have to leave time for that.

We intend to be proactive. Candidates will be identified more than six months prior to expiration. We'll be comprehensive. All reference materials, both the antisera and the extracts, will be updated.

One of our anticipated problems is that we're going to be spending a lot of time and money doing this. This is a big process. We have a lot of reference materials that we have to go through and maintain, and one of the possible solutions that we're hoping to study over the next couple of years is trying to switch at least some of our products over to lyophilized references, which will have a longer storage time, or another possibility that I'm not going to discuss is the possibility of investigating ELISAs based on serum pools. Again, taking advantage of the extreme stability of antibody solutions that are lyophilized for long-term storage.

So one of our plans is that as we go through this reference replacement that you have the details of, we're going to actually buy some extra reference material, more than we would ordinarily think we would need, to

either lyophilize ourselves or to have the manufacturers, if they're capable of doing it, to lyophilize for us, and then over the next year, we will begin to assess the stability and reliability of these lyophilized products compared with the more standard glycerinated products that we will be using. Then we will report out the results and distribute samples to the APMA membership prior to action. Obviously, not an action item this year or perhaps not even next year. We've really got a lot of studying to do between now and then, but we are hoping to have some information that will be helpful and will help our operations.

Now, I want to stop for a moment and explain the next few slides before we start. At the end of my presentation later this morning, I will be talking about specific regulatory proposals that we've actually put a fair amount of thought into, we think make good sense, and we'd like some feedback from you on them.

These next few slides do not fall into that category. These are issues that, frankly, I didn't spend much time thinking about before I came to this job at the end of August. I think these are questions that are going to be important for our operation probably not this year, probably not next year, but perhaps the year after.

They're hard questions. They're not questions

that are particularly easy, and I certainly am not putting together a comprehensive review. I will express my temporary opinion on the answers to these questions, but I want the committee to help me over the next couple of years in terms of starting to think about these questions, and I think they are important questions that we need to begin to address.

Basically, these issues are should CBER continue to be the source of reference standard allergens and antisera? And how should our standardization program continue?

Now, given the dynamite involved in both of those questions, you'll be surprised at how few slides I'm going to show, but I really want to drive the point home that these are things that I wanted very much to introduce today without really inviting specific answers today. I really wanted you to start thinking about them. I want you to help me through this over the next few years.

How do we manage our reference materials at this point? Well, this is the paradigm that we use. We identify a candidate reference. We do in-house testing. We send out samples to manufacturers for testing. Then, depending on the material it is, we purchase between a one and three-year supply and distribute it as the manufacturer has requested as time goes on.

What are the advantages of our being the sole source of U.S. reference materials? Well, obviously, the main advantage is control. We have the references, we know exactly what they are, and we are in charge of managing the references. We certainly can monitor them, and we do monitor them, as I said, every six months.

There's also an issue of fairness. We can insure that all the manufacturers get them as they need them. If rationing is necessary, we can assure that we do it on a fair basis, and also, and I think this is very important, that with us being the source of the materials, we can insure that qualified investigators who need these materials can get them as they need them.

What are the disadvantages? Well, frankly, the major disadvantages are inventory management and cost. Inventory management actually in the six months that I've been here has been a major headache. There's a disparity among the manufacturers at the rate of consumption of these products. As we've gotten towards the end of a product, we've actually had situations in which we've had to refuse to give out product or give a fifth or a tenth of what has been requested. These are not good situations for the manufacturers and they're not good situations for us.

One of the things that I've requested is that manufacturers give us an idea what their consumption of

product is going to be prospectively over the next year. That requires them to give some serious thought to how they're going to be using it and it lets us budget our materials appropriately.

In addition, and again, this is not a major issue by any stretch of the imagination, but I'm not going to make believe that cost is not an issue. It is. We are going to be spending upwards of $35,000 this year on this reference replacement program, so it's a significant investment, and it's an investment that I think will continue. But again, I think the major disadvantage is inventory management, more than anything else.

As I look at this and as I think about this in February, 1999, I think we need to continue doing exactly what we're doing. I think there's no good justification for changing our current status, which is to continue in our current role, and to work hard to upgrade our reference stocks and to evaluate better methods of maintaining our inventory, and that is what we are actively involved in now.

But I really wanted to sort of open the door for the committee to start thinking creatively, as I hope to be thinking creatively over the next year, about alternative paradigms and alternative ways that we can fulfill this very important function.

I also wanted to open the door on starting to talk again about standardization. Now, the current, highly successful paradigm for standardization is based on certain assumptions. Obviously, the current paradigm is based on the use of heterogeneous products. We're not talking about pure cloned products, for the most part. We're talking about heterogeneous, natural products that are largely glycosylated naturally, that are intact proteins, and, most importantly, in which there is a correlation between allergenicity, as reflected in skin testing and competitive ELISAs, and immunomodulatory activity, which is the therapeutic function for which many of these products are intended.

Our current standardization targets, according to the recommendation of the advisory committee last year, are, in this order, latex, cockroach, and tree pollens. We are actively working on latex. We are going to be actively working on cockroach, which arguably is a significant public health issue, and then tree pollens will follow. So we are pursuing standardization efforts.

But, just to take latex at random as an interesting possibility, the idea of standardizing latex is a little bit of a daunting one. There have been many identified allergens for latex. Now, it's extremely unlikely that all of these are equally important. It's

also extremely unlikely that most of these are important at all. Many of them probably are relatively minor, but the fact is we really don't know with great certainty what the major and minor antigens are for a large portion of the population that are latex-allergic, and so the study of standardizing a latex product is I think perhaps more scientifically difficult than some of the standardization efforts that have gone on so far.

Furthermore, although we are in a position to assess a latex extract for its content of perhaps six or seven of these, we certainly are not in a situation where we can analyze all of these in a particular latex extract.

What are the limitations of the current standardization paradigm? The limitation mainly, as far as I'm concerned, is that it is of uncertain predictive value for peptides, plasmids, modified allergens, and other, possibly non-glycosylated, products. These are products that we anticipate we're going to be seeing down the line over the next five or six years, and the current methods that we use to assess an allergen and to monitor it and to do our quality control simply are not going to apply to many of these products.

Again, definitely not the major issue. Cost is not the major issue, but it is an issue that I don't think we can completely ignore, and that is that the paradigm is

that the cost of the standardization is largely borne by the FDA.

Now, in case you wonder whether I'm being too futuristic thinking about cloned products, I think that cloned products for allergen immunotherapy are right around the corner, and what I think drives this is not just the science, but very much the economics of cloned products. Immunotherapy doses typically are between 10 and 30 micrograms a month once you get up to maintenance therapy for effective immunotherapy. How much would that cost, let's say, if you were doing immunotherapy with any of the current commercially available cloned products?

Well, just taking three, filgrastim, or Neupogen, is derived from E. coli, it's 175 amino acids, and it costs all of 53 cents a microgram retail. That actually I think is very accessible in terms of the cost of immunotherapy. Okay, filgrastim is cloned in E. coli, we know it's non-glycosylated, and let's go to another product.

Sargramostim is cloned in yeast, partially glycosylated, 127 residues. It's cheaper. It's 52 cents a microgram.

Okay, we know that yeast don't really glycosylate quite as well as mammalian cells. Let's look at erythropoietin alpha. It's grown in Chinese hamster

ovary cells, 165 residues, extensively glycosylated. It comes out to be considerably more expensive, $1.55 per microgram.

But again, you can see the point that I'm making here, and that is the economics of these cloned products I think, and the science of the cloned products, really leads us inescapably to the fact that we are going to be seeing submissions of these products in the near future and we need to develop paradigms that we're going to use to assess them.

Unfortunately, the existing alternative approaches are clearly not satisfactory. Again, this is an example where I'm not going to be giving you the answer. I'm really trying to start a discussion with you about what direction we should go.

One possibility is something called consistency monitoring, in which a manufacturer will set up its own internal standard and make sure that the product continues to be consistent relative to that standard. That actually is attractive for many purposes, and you can imagine many situations in which you would recommend consistency monitoring, but the problem with that is that there's really no industry standard at that point, and that, although you've insured some kind of consistency, you still have to determine what parameters you're going to want to

make consistent and you really need to worry then about the products that are made by two or three different manufacturers within the industry.

We've already had some discussion about the possibility of looking at pure allergens, perhaps with a monoclonal antibody, perhaps with a monospecific polyclonal antibody. Again, the problem is that we really can't state with confidence for a number of allergens whether all the component allergens have been identified or characterized.

Finally, we can use other methods of in vitro characterization, but the problem is that we have not at this point established good criteria, and I think that's probably one of the areas that I'm going to be spending some time focusing on, and that is, if we're going to use other in vitro methods to characterize these products, what criteria are we interested in establishing?

Again, as before, we are pushing forward with the current standardization program. We plan to complete the laboratory portion of latex standardization really within the next six months, probably sooner. I think we've gone a long way towards that, and we're going to move relatively quickly at this point.

We do intend to initiate work within the next six to 12 months on cockroach standardization. Again, my sense, from reading the advisory committee's transcripts

from last year and also my own personal opinion, is that from a public health standpoint cockroach is a more urgent product to standardize than tree pollen is.

One of the themes I'm going to come back to is that what I'm telling you about today is ambitious, and I know it's ambitious. I wouldn't be telling you all the things that I'd like to do if I didn't think that I have the support of the Office of Vaccines and the Division of Allergenic Products behind me.

We have at this time a lab chief. That's me, and I am planning on staying in this position for a long time, so hopefully it will be stable. At least, from my point of view, it will be stable.

We have Dr. Soldatova, our postdoc. She is going to be staying with us. We have two biologists currently working, Maneesha Solanki and Beth Paupore. We have hired a new biologist. This is a slide I just made up over the weekend. He's going to be starting on March 1st. So we are going to be up to a third biologist, and we are recruiting a fourth one.

I think once we get that fourth biologist, we are really going to be at a full complement to serve the regulatory function that I'm going to be describing, and also to do some of the important research that we'll be talking about in the next hour.

That's it.

DR. OWNBY: Any of the committee members have questions for Dr. Slater?

DR. WRAY: Yes. Please go back to your replacement of new reference extracts, where, for example, mite S3 was replaced by S4.

DR. SLATER: Yes.

DR. WRAY: I understand S3 was probably outdated, so to speak. Is that a consistent -- was there a change in the potency there?

DR. SLATER: When we replaced mite S3 with mite S4, we did have some concerns by the manufacturers that there was a problem with using S4, and I actually almost put that exchange of memos in your packet to show you. I'm sorry now that I didn't.

One of the immediate problems that the manufacturers reported back to us was that S4 seemed to have a lower titer than S3 did, so they had to use a lot more of it. One of the features that then came out as they were using less and less dilute sera was the background was coming up and the working range that they were working in was really unacceptable.

The initial communications that we had from the manufacturers were in November. We got back to them -- I don't remember whether it was December or early January --

with some suggestions as to how to modify the assay further to improve the baseline. Our reading of their data was that we saw the problem as mainly a baseline problem. We actually were successful in-house at using a relatively dilute solution of the S4 product, and we recommended that they go back and reoptimize the assay using a lower concentration to see if they really needed to get up to the higher concentration of the serum.

In terms of the specificities of the extract, using immunoblot of isoelectric-focused proteins, we found that it was equivalent, and in fact -- I don't remember how many sera are in that product. I think there are six sera. Three of them were identical to what was in the previous one, so there's considerable overlap between the two products.

The reason I was going to include it is that it's a typical example of some of the day-to-day interactions that we have with the manufacturers that I actually think are good. I mean, I think those are positive interactions when there's a specific problem with one of our references and we can try to troubleshoot it and see if we can improve things for them.

DR. WRAY: Of course, I'm concerned as a clinician to be sure when I'm getting another batch that --

DR. SLATER: Well, that's right, but whenever

we switch from one extract to another, and from one serum to another, we do the kind of analysis that I showed you with the latex, and that is we look at the relative potency of the products that we have in-house.

In fact, one of the specific questions that one of our manufacturers raised was that when they tried to replicate our results using our standard, they also found that the two reference antisera were equivalent. It was when they took one of their products, their in-house products, and analyzed it that they actually saw about a 15 to 20 percent difference, and that was what we were concerned about. We were wondering whether perhaps the background problem was more significant with some extracts than others.

DR. WRAY: Thank you.

DR. SLATER: But it's a question that we take very seriously and we try to answer it as quickly as we can.

DR. EIN: Well, just to follow up on that, some of the manufacturers and some of the clinicians had the sense that with this change in standards there were increasing numbers of reactions to immunotherapy, and is there any mechanism that you have -- I'm not aware of any -- by which you can get that sort of feedback?

DR. SLATER: I'm sorry. Is there any mechanism

question really has to do in the end with what is the sigma or standard deviation of the products that are actually sent to us? How does it compare to the sigma of the assay?

Just thinking about it conceptually, if in fact the sigma of the products is small and they're sending us products that are fairly tight as a consequence of the manufacturing techniques, the quality control that goes on before they send products to us, if they send us products that are pretty tight around a relative potency of 1, and our assay is pretty broad in terms of its relative potency, well, then we're really not so concerned about the average product. We just want to make sure that the outliers are cut out. We want to make sure that we lose those outliers.

So we want to look at the relationship between the sigma of the products that we are sent and the sigma of our ability to look at those products and, assuming a Gaussian distribution, the sigma of the observed products equals the sum of the sigma of CBER's assay and of the manufacturers' products, and what we're looking for is this.

Well, how do we look for that? Well, we can make an estimate of what our observed sigma is. If you look at from 1995 to 1997, we rejected 53 out of 414 products that were sent to us in 1995 to 1997, or about 13 percent of the extracts failed. That translates into an

by which we can get that feedback?

DR. EIN: The kind of feedback that the changes in references or that the materials that are going out seem to be associated with more reactions. I mean, it's one thing to look at reference sera. It's another to look at what actually happens when these products are used clinically.

DR. SLATER: Those are data that we're terribly interested in seeing. I haven't seen any of those data, but certainly, if we are confronted with those kinds of data, I think we would have to reevaluate what we were doing in terms of the extract. That's obviously very important.

Paul?

DR. TURKELTAUB: If I could just make one comment to Dan's comment, if there's some concern about drift in potency from lot to lot, side-by-side skin testing is a very easy way to know whether there is a difference in potency, which is somewhat low tech, but very germane to answering the question, and if there could be that kind of effort made among the professional organizations and manufacturers, that would be a very simple way, as opposed to going to this spontaneous reporting system about adverse events, because everybody handles these products differently in dose regimens, et cetera, et cetera.

DR. OWNBY: Any other questions from the committee?

(No response.)

DR. OWNBY: We have a few minutes. Any questions from the other members of the FDA who are here?

(No response.)

DR. OWNBY: If not, we're just a couple of minutes ahead of schedule, and I think we can go ahead and take a break if everyone will be back so we can start promptly at 9:35, which I believe is on your agenda as our next start time.

(Recess.)

DR. OWNBY: Well, we're continuing on with Dr. Slater's report on the research in the laboratory.

DR. SLATER: Thank you.

Committee members, in your prepackage, the package you got a couple of weeks ago, you have a bibliography from the lab and you have five reprints and one preprint, and that's going to be the subject of much of what we say for the next 45 minutes or so.

It is my intent for this laboratory to have an extremely active research program, and I think, as I closed in the previous talk, with a full complement of a lab chief, a postdoc, and four biologists, we will be able to perform the regulatory function that was the subject of the

first portion of the talk and the research function that we're going to be talking about over the next 45 minutes or so.

But I want to stress one important point that I am going to be bringing up over and over again, and that is that it is my intent to make the research program of this lab so clearly identifiable with our regulatory function that we will not really have that kind of a clean division between regulatory and research. All of the research activities that I think we should be performing, and I hope we will be performing, are going to have direct relevance to the regulatory activities of the laboratory.

The way I have formulated it so far is to have two broad functions, two broad research questions that we would like to answer. One relates to allergen structure and function, and the other to immunomodulation.

Under allergen structure and function, we need to focus on issues related to glycosylation and allergenicity, enzyme activity and allergenicity, and better identification methods of the allergens that we need to regulate.

In terms of immunomodulation, we would like to explore further some of the work that's already been done with epitope-specific immunotherapy, the use of DNA vaccines, the possible role of lipopolysaccharide in

allergenicity of products, and issues of cross-sensitization between allergens and related allergens.

There are six references on the list that you have. The first three appear here. One was from Dr. Lin and Theresa Liu. This is the paper that we discussed in the first hour on the epitope stability of Group 1 and Group 2 allergens. This appeared in the Annals of Allergy last year, and I'm not going to be discussing this manuscript any further.

Dr. Soldatova and colleagues published an excellent paper in the JACI a few months ago on the superior biologic activity of recombinant bee venom allergen hyaluronidase expressed in baculovirus-infected cells as compared with E. coli. This paper in many ways touches upon some of the more important issues that I'd like to continue to investigate, and I'm actually going to talk about that study in some detail.

Somewhat more briefly, I will refer to a paper that appeared in the Journal of Biological Chemistry in the fall that was authored in part by Li-Shan Hsieh, who is the Ph.D. that used to work in the lab. Some of this work was done in our lab with Akira Akasawa and also Brian Martin, who's also at FDA. This was a study of the cloning of an avocado allergen and some of the things that they found with that.

In addition, I've listed three papers that we authored from our work at Children's, and this was work done by Beth Paupore in my lab at Children's. Two of these appeared in publication in the JACI and one of them has been accepted, but by Molecular Immunology, and will be published in either the March or April issue. These are not studies that were done at LIB and FDA, but I wanted to talk about these to give you an idea of what I have done and some of the work that I would like to continue in LIB.

In terms of the first broad area of interest, allergen structure and function, I'd like to focus on glycosylation, and from the first talk that I gave, it's clear where I'm coming from in terms of studying glycosylation. I think that much of our regulatory function is going to be tied into issues of the glycosylation of allergens.

Some of the questions that I'd like to ask are is the decreased antibody binding of non-glycosylated antigens primarily a function of impaired folding? What is the biochemical anatomy of the glycosylation requirement for an antigen to have good interaction with antibody? Can non-glycosylated allergens equal native allergens in immunotherapy? And, most importantly, how can non-glycosylated products be evaluated for diagnosis and therapy?

In terms of enzyme activity, we are going to be focusing somewhat on what is the relationship between enzyme activity and allergenicity with respect to antibody binding, bioavailability, antigen processing, and, obviously, the specific regulatory applications have to do with hymenoptera, dust mites, and latex, but also other allergens as well.

DR. SAXON: I'm a little confused. What did you mean, enzyme activity? That's specific activity related to --

DR. OWNBY: Microphone.

DR. SAXON: Sorry. I'm almost trainable.

What do you mean, the enzyme activity of latex is related to its antibody binding? I'm not sure what you meant.

DR. SLATER: Well, there is no evidence yet about latex, but obviously, with hymenoptera, the enzyme activity, hyaluronidase and phospholipase, is used as a measure of the integrity of the allergens, and there's enzyme activity in dust mites as well. We'd like to see what the actual role of enzyme activity is in the various things that we're concerned about in terms of immunogenicity. Theoretically, at least, an enzyme that's an allergen could lose all of its enzyme activity and still be perfectly allergenic, perfectly functional, but that

really hasn't been worked out for a number of the allergens that we're concerned with.

DR. SAXON: Okay. I wasn't sure what you meant by this slide.

DR. SLATER: The answer may be unrelated. It probably isn't unrelated, and I'll show you some data that suggests that.

DR. SAXON: I thought you were pursuing this idea that some people have proposed that most allergens are enzymes, and therefore chew on cell surfaces, and it has not really held up.

DR. SLATER: Right.

DR. SAXON: That's not what you're saying here.

DR. SLATER: No, it's not primarily my question, although the issue of bioavailability in antigen processing certainly would tie into that, but certainly I'm not going to show you any evidence that that has any bearing on these two points at all, but rather issues having to do with antibody binding.

Yes, sir?

DR. KING: Jay, I just want to continue what Andy just said. You know, even the phospholipase, people have made one that removed the active site and it was perfectly active, so it's not necessarily related.

DR. SLATER: Right, and that's the kind of

study that we'd like to look at with other allergens.

DR. KING: Then I'd like to also comment about your raising the issue about the role of glycosylation and allergenicity, because again, as you know, bee venom phospholipase can be isolated in glycosylated and non-glycosylated or very poorly glycosylated form, and they don't show much difference in allergenicity. The percentage of antibodies that are directed toward the carbohydrate portion is actually very, very small.

The other thing, you know, the report of cross-reaction of bee venom with some different plant material is due to the carbohydrate portion. So it can form an IgE epitope, but it's not of the dominant one.

DR. SLATER: That's right, and I think one of the themes in the first two papers that I'll discuss is that the answer probably depends, and identifying the actual mechanism by which it depends, in what kinds of allergens the glycosylation is important and what kinds you would expect it not to be important, I think is an important thing to look at, so that we can actually have perhaps a predictive model of what would be worth looking at more carefully.

DR. SAXON: I want to add another part to that glycosylation. There were some very nice studies looking at nut cross-reactivity, as I recall, from Europe showing

that in fact nut cross-reactivity in in vitro tests that was anti-carbohydrate was clinically irrelevant, but accounted for the majority of the in vitro cross-reactivity, and I think that's something very important to pursue when the FDA and we get involved in assays to diagnosis diseases which may be in fact picking up carbohydrate binding which is clinically not very important. I think it's something that also has good background already for it in the nut story.

DR. SLATER: Good.

So again, the first paper that I'm just going to review quickly was authored by Dr. Soldatova and that appeared in JACI in 1998. They cloned and expressed bee venom hyaluronidase. They cloned it using these primers, and cloned it into several vectors, including two different E. coli-based expression systems, one of which had the tag on the N portion, the other of which had the tag on the C portion, in addition to cloning it into a baculovirus-based expression system.

When they looked at antibody binding, and this is the binding of individual patients' sera to hyaluronidase by Western blot, the first strip of each grouping is negative control serum and then the second, third, fourth, and fifth strips are individual sera from allergic patients who are bee venom-allergic and have

antibodies to hyaluronidase.

The first grouping, A, is the native purified hyaluronidase, and this is sort of your baseline that you're looking at. Again, a negative control and four individuals that had significant visible binding to that protein.

The two E. coli preparations of hyaluronidase, one with the histidine tag on the N portion, the other with the histidine tag on the C portion, show some binding. Again, this is the negative control, and these are the four antisera, the same ones used in the first grouping.

Interestingly, in the N portion you see some incomplete expression, and that's one of the reasons they switched in this study to the C terminal his tag, and you see some binding, but it seems to be quantitatively somewhat less. You can't really tell from this, and we'll look at some other studies in the next slide that will show quantitatively what the difference was.

This is the baculovirus-expressed product, and again, you see some uptake, probably roughly the same as the native material, but perhaps a little bit less on this Western blot.

Quantitatively, you can look at the same question using RAST inhibition, and here you see in the open squares the RAST inhibition with the E. coli-produced

Class 2, nine Class 3, and three had a Class 4. So again, evidence that the baculovirus-expressed hyaluronidase had a comparable IgE binding to the native hyaluronidase.

When they looked at specific hyaluronidase activity, they found something very similar, and that is that the baculovirus product and the native product were indistinguishable in terms of their specific activity, units per milligram, whereas the E. coli product had a significantly lower specific activity.

Notice that the bee venom has much lower specific activity, but that's because it's not a pure product and there are a lot of other proteins that are going into the denominator.

So the conclusions from that study were that obviously honey bee hyaluronidase had been expressed, and for enzyme activity the native product was roughly equal to the baculovirus product, which was significantly greater than the E. coli product. For IgE binding, the same kind of relationship applies. Native equal to baculovirus, much greater than the E. coli preparation.

Dr. Soldatova has continued to study bee venom allergens and her current area of interest is in looking at acid phosphatase. This is information that is very new and very fresh, and I can't actually show you the data itself, but she actually cloned acid phosphatase from bee venom

using cDNA with primers that she determined from a genomic sequence that she had previously cloned out. About half of the putative sequence has been identified, and what's most exciting about it is that it has a very strong homology to the other cloned sequence, insect acid phosphatase, that is from Drosophila, but almost no homology at all to either mammalian acid phosphatase or Leishmania acid phosphatase.

So we really think we do have a bona fide insect acid phosphatase. Obviously, Dr. Soldatova has a lot more work to do, because she has to continue the internal sequencing. She also has to do five prime and three prime rates to get out to the ends of the sequence, but we're hoping to be able to do some studies with the acid phosphatase that she has cloned out. She's also going to be turning some attention to Allergen C in the near future.

Briefly, talking about the other study that I wanted to refer to early in this talk, and that is the study authored by Sowka and Li-Shan Hsieh, who, again, is the Ph.D. now at CDER who spent some time working in our lab on the cloning of Prs a 1, an endochitinase and major allergen of avocado, and its expression in yeast.

It turns out that one of the major allergens in avocado is a 30-kilodalton protein. What you see here is crude extracts of avocado run on a Western blot with 20

sera from allergic patients, the normal negative control serum, and you see that most -- not all, but most of these patients -- have a significant band at about 30 kilodaltons.

The early part of this study, it's entirely their strategy to purify that protein and to also clone it out from cDNA, and I will skip over that, but when they run gels with the purified native Prs a 1 and the recombinant Prs a 1 on SDS-PAGE, they get something that looks almost identical in terms of its migration on SDS-PAGE.

Furthermore, they were able to show that there were significant sequence homologies of Prs a 1 with prohevein, and some smaller homologies with banana chitinase and the latex-based chitinase as well.

IgE from allergic patients binds to the recombinant Prs a 1 at least as well as it does to the native Prs a 1. Now, remember this recombinant was not an E. coli. It was in yeast. In yeast, there is some glycosylation. It's definitely not equivalent to mammalian glycosylation, but apparently this is a situation in which even the inadequate glycosylation of yeast is enough for what seems to be perfectly adequate IgE binding.

Furthermore, they were able to show that there was inhibition of IgE binding to native Prs a 1 using the recombinant product. This is basically a blot in which

native Prs a 1 was run. You see the pickup here on the 30-kilodalton band that is inhibited by preincubating the serum with recombinant Prs a 1.

So in their study, they were able to show that Prs a 1 was cloned and sequenced. They found that the natural product and the recombinant product -- again, recombinant in yeast this time -- had equivalent IgE binding. What I didn't show from this paper is that they also had equivalent endochitinase activity, and it also had equivalent fungicidal activity in an in vitro assay as well. So functionally, as well as with IgE binding, this recombinant product in yeast appears to be, for all intents and purposes, equivalent to the native Prs a 1.

So again, getting back to your initial question, I think it does vary from antigen to antigen, and we certainly would need to look at this antigen in a completely deglycosylated state and see whether it was equivalent as well, but it may well be.

Additional questions that I think are raised by these two papers are these. If an allergen that is not glycosylated, glycosylated abnormally, or denatured shows poor IgE binding or impaired enzymatic activity, how can we go about evaluating it as an immunotherapeutic reagent? And now I'm sort of shifting gears and pushing us into our regulatory hat, and that is what kind of methods can we use

to evaluate these products as they come along on an ongoing control quality basis?

The identification methods that we currently use for allergen structure include SDS-PAGE, isoelectric focusing, which doesn't appear on this slide, and immunoblotting, and these can be quantified. In other words, you can do densitometric scans of these to try to quantify them. We don't quantify them at this time, but we certainly could attempt to do that.

But one area of interest over the last several -- actually, over the last year in the Division of Allergenic Products has been the exploration of the possible role of MALDI-TOF techniques to better identify allergen structure in a more fine structural method.

This is a horrible slide that I'm going to skip over. I will skip to something that's much clearer.

MALDI-TOF stands for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The reason it's on the slide is if it weren't on the slide, I would have had to have memorized it. So now I can just read it off the slide.

Basically, this is an attempt to do mass spec analysis of proteins using a method that works well for mixtures of proteins. The standard electrospray method doesn't appear to work well for mixtures. This one has the

potential, at least, for working well for mixtures.

In this preparation, you take a sample of a mixture of proteins, you mix it with a matrix of a UV-absorbing compound, and dry it on a sample plate. You then place that sample plate in the unit. This is a unit that's maintained under a high vacuum. Then the sample is bombarded by a laser at time zero.

When the laser hits the sample, the sample is ionized, and desorption occurs, and the ions, both positive and negative, are brought down the tube -- there's a beam guide that brings the ions down the tube at rapid speed -- and it hits a detector. The time between the impact of the laser on the sample and the impact of the particles at the detector is called the time of flight, and the samples, as you can imagine, will travel more slowly the larger they are, and they will also travel more rapidly the higher the charge. So the time of flight is related to the mass divided by the charge, or M over Z.

This is very initial data, but we actually did MALDI-TOF analyses of various venom proteins in an attempt to develop a model to quantify and to look carefully at some of these products. The top MALDI-TOF tracing that you see is of mellitin, and mellitin is a very important protein in bee venom. It constitutes about 50 percent of the dry weight of the bee venom.

What you see here is a major peak of mellitin at just about the predicted molecular weight of 2,800. You also see another smaller peak that's at exactly half of the predicted molecular weight. Well, that actually is easy to explain. This is a double-charged unit, so whereas this, the main part of the sample, has a single charge and comes out as an M over Z of 2,800, this is double-charged and comes out as exactly half. Very nice, simple, straightforward, needle-like peak, just what you'd like to see.

Unfortunately, in the real world, such as the real world of phospholipase -- this is a tracing of native phospholipase. There are at least three native forms. This was a product obtained from Sigma, and it has multiple glycosylation patterns, and, as you can see, there are several peaks, all around the predicted molecular weight of about 1,600.

But again, not quite the sort of fine peak that you would hope to see, but you can see here that there is a potential at least, if you can spread out the axis, of really identifying the different glycosylated forms and the different molecular forms of this, and I think this could be a potentially very good tool for looking at this particular protein.

Likewise, with hyaluronidase, about a molecular

weight of 43,000, which is just where we would expect to see it. This is the baculovirus-expression product, and again, there are probably multiple glycosylated forms in here in addition to some isoforms that have as many as four or nine fewer amino acids on the N terminal, which is why this is spread out so much. But again, if we spread out the axis, we should be able to see more. Likewise, with acid phosphatase, another glycosylated native protein, we see this kind of spread.

Finally, Allergen C, which is a large molecular weight protein, over 90 to 95,000. This is a native product that's probably glycosylated, although we're not sure, and again, we're hoping to be able to get some finer detail on this.

When we run a whole bee venom over the product, we get a very large mellitin peak, we get a very large phospholipase peak. Again, not particularly surprising, given the amount of these products in bee venom. We also get a number of smaller peaks, and we're trying to look at ways of desorbing out these major proteins that take a lot of the desorption energy, and seeing if we can identify the smaller peaks.

Now, the purpose of all this is to try to develop a quantitative profile of natural allergen preparations, and the question obviously is can we use

MALDI-TOF method to carefully assess the glycosylation of recombinant allergens? This is what we're going to be looking at over the next year, starting with bee venom allergens, because we think it's a good paradigm, we know the allergens, we know what's involved, and Dr. Soldatova will be spearheading this effort.

The next series of studies are ones that we actually did at Children's Hospital looking at the potential for epitope-specific therapy of the latex allergen Hev b 5. We hope to do a good, complete human epitope analysis of Hev b 5, and potentially be involved in support for future clinical trials of latex immunotherapeutic reagents.

The idea behind epitope-specific immunotherapy is to identify and purify antigen, identify the T-cell epitopes of the antigen, identify B-cell epitopes or the IgE binding sites of the antigen, and to administer immunotherapy with the T-cell epitopes.

What I'm going to be showing you now is from a preprint that was included in your packet on murine B-cell and T-cell epitopes of Hev b 5 from natural rubber latex. This is the paper that Beth and I wrote. Our co-author in Australia was Robin O'Hehir, and we're going to be publishing this in Molecular Immunology next month or in April.

Very briefly, we were able to show with mice that were immunized to Hev b 5 specific peptide inhibition of binding with specific peptides, and from several different mice we have really a very similar pattern of binding to peptides 75, 79, and 85.

In addition, we were trying to identify the T-cell epitopes in spleen cell preparations from these mice. These were at largely different locations, and from studies using several mice, both for the B-cell and the T-cell studies, we were able to construct an epitope analysis map of Hev b 5. The alpha refers to alphahelical regions of the protein, of which there are only a few. The Bs and the stars represent B-cell epitopes by different methods with different mice, and then these circles with the stars in them, these very prominent-looking stars, actually are the T-cell binding regions that were identified from numerous studies.

What you can see here is that there are large T-cell binding regions, at least two of them, that have no IgE binding activity in any of the mice. So we were going to use this to pursue epitope studies of treatment in Hev b 5-sensitized mice, and also to tie it into some of our DNA vaccine work that I'm going to be talking about in a few minutes.

Interestingly, at just about the same time, our

collaborator, Robin O'Hehir, and her associate, Jennifer Rolland, are now beginning to look at Hev b 5 epitopes in Australian health care workers with latex allergy. In her studies -- and this is all data that she faxed to me literally a week ago, so this is very early data -- she was able to clone out latex reactive T-cells, and then study their epitope specificity for Hev b 5. What she was able to find is several identifiable epitopes that recognize specific Hev b 5 fragments in these two patients. Obviously, she's going to be continuing these studies with other patients.

So we have identified some putative B-cell and T-cell epitopes in mice for Hev b 5. We have a preliminary identification of possible human T-cell epitopes suggesting dominance. In other words, agreement between the human and the mice studies in these specific peptide regions.

We will be pursuing some additional studies in mice as a possible mode for immunotherapy. My plan is not to proceed at this point with studies using these specific epitopes, but rather to put this study into the context of some of the work that we're going to be doing with DNA vaccines that I'm going to describe next.

Yes, sir?

DR. UMETSU: Do these T-cell epitopes differ if the strain of mice differ or in humans?

DR. SLATER: That's one of the other things we need to look at. We have not looked at that yet. We've only done this with BALB/c mice. We would not be surprised if they were different.

DR. UMETSU: How about in Robin O'Hehir's data, where she looks at the T-cell epitopes from different people? How many different people has she looked at?

DR. SLATER: She has collected 20 Australian health care workers that are Hev b 5-reactive at this point. She's spent much of the last six months working out the method for growing out the right T-cell clones to be able to study this. She had significant problems with maltose binding protein-recognizing clones, as opposed to Hev b 5-recognizing clones, and what she found is that when she grew up the clones in the presence of a latex extract first, she was then able to come back with Hev b 5 and get Hev b 5-specific clones grown.

DR. UMETSU: So in those 20 individuals, there is an immunodominant T-cell epitope? Or two, as it looks like on that slide?

DR. SLATER: She's only worked with two since she figured out how to grow out the cells.

DR. UMETSU: Two people or two --

DR. SLATER: Two patients.

DR. UMETSU: Two patients.

DR. SLATER: Right, people. It's very preliminary work.

Another area that we started investigating about a year and a half ago at Children's was the possibility of using DNA vaccines for allergen immunotherapy. DNA vaccines are plasmids containing an encoded region -- the encoded region in this case would be the allergen of interest -- that when injected or taken up by muscle cells or other cells, the proteins are expressed in vivo and are released, causing an immune response.

All of the initial work with DNA vaccines was done as an effort to raise immune responses to infectious agents, but starting in the mid-1990s, work really showed fairly convincingly that DNA vaccines could potentially be used for the reduction of IgE responses, both by Raz and his colleagues in a paper in PNAS, and subsequently using beta galactosidase as the antigen in mice, and by Hsu and colleagues in Nature Medicine in the subsequent paper in both rats and mice using Der p 5 as the antigen.

In both of these studies, they were able to show that IgE responses could be blunted when the mice were given DNA vaccines in advance or they could be reduced when the DNA vaccines were given after exposure and sensitization to the antigens by classical methods.

The advantage of DNA vaccine-based

immunotherapy is that under certain conditions DNA vaccines appear to give a fairly consistent Th1-specific response. Most attractive, however, is that there is prolonged expression of the antigen, at least four to six months, and, theoretically at least, multiple antigens can be encoded on a single plasmid.

However, there are problems, most of them theoretical, and that is obviously DNA vaccines have an unproved safety profile. There is concern regarding mutagenesis. There is concern regarding the tissue specificity of the plasmid, the rate of allergen release and the kinds of reactions that might occur, and also the possibility of adverse responses due to CD8 responses to the antigen.

The thing that we're most concerned about is the control of responses in vivo. In other words, once you inject the plasmid and it is taken up, as has been demonstrated for long periods of time, how do we control the responses that occur? In particular, we're very concerned about the control of these responses because of some preliminary experience that we had with a Hev b 5 DNA vaccine that we constructed.

In particular, we found that the sense construct -- the construct that contained Hev b 5 and the sense direction was actually expressing Hev b 5 -- was

highly toxic to presensitized mice when we injected it into the tongue. The reason we selected the tongue is it's a place where you can do an intramuscular injection under direct visualization. The toxicity was not secondary to trauma to the tongue. Rather, it occurred three to five days after injection, and these mice actually got very sick.

Mice injected with the identical plasmid, but who had not been presensitized, were fine. Mice injected with the antisense plasmid, whether they were presensitized or not, were fine as well. It was only the mice that were presensitized with Hev b 5 protein who were injected with the sense plasmid that seemed to have a significant problem when they were injected with the vaccine. Interestingly, when we injected the construct intradermally at the base of the tail, we did not have any toxicity.

Yes?

DR. SAXON: Jay, what do you mean toxicity? You used the word "toxic." Do you want to explain a little bit more what that is?

DR. SLATER: They developed a severe local inflammatory reaction. They became systemically ill. None of them died. They all recovered, but their recovery period took four to five days, during which time some of them looked like they were going to die. But it was a

local inflammatory reaction, and it's certainly possible that all of their systemic symptoms were just due to that local inflammatory reaction, but it was very impressive, and on an experimental basis it was very specific.

The results that I'm going to show you now were actually published in JACI just a few months ago. The latex allergen Hev b 5 transcript is widely distributed after subcutaneous injection in mice. The purpose of this was really just to look at what happens when you inject DNA vaccine to an easily traceable transcript when you inject it at the base of the tail, which is sort of a standard place of injection.

Our results basically are summarized in this one slide that's a little bit complicated. This is looking at the transcript by RT-PCR for Hev b 5. What we did was we injected mice and then three days, seven days, and 14 days after injection, we sacrificed the mice, and took tissues from different parts of them to look for the presence of the transcript by RT-PCR.

Again, these are in groupings in the top, and one grouping and another grouping here at the bottom. These first four are tissue taken from the actual site at the base of the tail. This is an uninjected mouse -- there's no signal here -- three days beforehand, seven days, and 14 days, and you see a very bright band, and I'll

show you that we were able to show that this was Hev b 5 in a subsequent experiment. So there seems to be persistence of the transcript at three and seven days out, and it seems to disappear or start to disappear by 14 days out after injection.

In the second grouping, which is from the dissected lymph nodes, and the third grouping, which is from the spleen, you see the same thing, except there seems to be perhaps some persistence at 14 days out, and that's not terribly surprising. You would expect this transcript to be picked up by immune-specific cells, and in fact that's what we see.

What we were a little surprised about was to see a faint signal in the lung tissue of these mice, and we were even more surprised at 14 days to be picking it up in the blood, just in blood taken from mice sacrificed 14 days out.

We did not find it in other tissues. We did not find it, for instance, in the tongue, but there seems to be some suggestion from this that when you inject one of these DNA vaccines with a good strong promoter, which is what we were using, you get wide dissemination of the transcript at different parts of the mouse.

We did a restriction analysis of the RT-PCR sequence in order to verify that it was in fact Hev b 5

that we were seeing. We used SfaN1, which is not a very common restriction enzyme, but we used that because it recognized the particular sequence in the Hev b 5 sequence that would cleave the 274 base pair sequence to 155 and 119, and in fact this is exactly what we saw. Here's our 274 sequence, and after cleavage, 119 and 155 were what we found.

So we would like to continue to study DNA vaccines for Hev b 5, but this time we would like to modify the approach and explore the possibility of looking at specific T-cell epitopes cloned into the DNA vaccine or, alternatively, to look at full Hev b 5 sequence, but using weak promoters or tissue-specific promoters as an approach to try to control the expression of the product and control the reaction that these mice actually have. We have a control system with a positive reaction that we can use to test out the efficacy of these approaches in reducing the nonspecific inflammatory response.

The next series of investigations also appeared in JACI, just actually two months ago, and that's an investigation of lipopolysaccharide's effect on IgG and IgE responses of mice to Hev b 5. The genesis of this project came from a report that appeared about two years ago from Brock Williams in the Annals of Allergy on the not terribly surprising observation that latex gloves have a significant

content of endotoxin, and they raised the possibility that perhaps this endotoxin might be affecting immune responses.

But they didn't come up with that idea out of the blue. In fact, there is a fairly rich, old literature that shows that LPS can affect IgE and IgG responses in mice in a way that might conceivably cause problems.

So we developed really a fairly straightforward and almost simpleminded protocol, and that was to look at mice, give them by the nasal route either saline, LPS, Hev b 5, or LPS in combination with Hev b 5. These were mice that were anesthetized with methoxyflurane, so they were able to inhale a fair amount of this, and we gave it to them in two courses. One was six doses over a period of two weeks, and then several weeks later, we gave them another three doses over a period of one week.

The results basically look like this, that after the first course, if you look at either the mice getting saline or Hev b 5 alone or LPS alone, the specific amount of anti-Hev b 5 IgE was unmeasurable using this assay. When we gave them LPS in combination with Hev b 5 -- this is after a single course -- we had a significant, measurable amount. This normalized titer here of about 1 means that the amount that those mice made was equivalent to the pooled sera from hyperimmunized mice that had a relatively high IgE content. So a significant amount after

only one course when we combined LPS and Hev b 5.

Now, after the second course, you see here that the mice getting Hev b 5 alone do start to have a measurable amount of IgE against Hev b 5, but again, the combined response was way ahead of the solo response. Then by the time we came back several weeks later, the specific response in the Hev b 5-alone mice seemed to have come down, and again, the error bar on this was fairly impressive, so this really wasn't very interpretable, but we certainly can show that after a single dose the LPS has a dramatic effect, and even after the second dose it has some effect.

DR. KING: Jay, may I interrupt you, please?

DR. SLATER: Absolutely.

DR. KING: Jay, on this slide, it shows that your measuring antibodies to your maltose binding protein have been conjugated. Is that right?

DR. SLATER: Yes, that is right.

DR. KING: So do you really ever check that? Does that really work with Hev b 5?

DR. SLATER: Yes. I'm not showing the data, but the thing that we can show is that we can show it two different ways. One is by Western blotting we can show that when you separate out the Hev b 5 and the maltose binding protein, it actually recognizes both, and it

clearly does recognize both.

Furthermore, in the T-cell studies that we did with these same mice, there's no doubt that some of the reaction is due to maltose binding protein, but again, we can separate it out. But it definitely reacts to both. That's true.

DR. KING: But then it really isn't -- you're measuring the immunogenicity of Hev b 5. You're measuring the conjugate.

DR. SLATER: This is true, but the effect of LPS is interesting whether it's on the conjugate either way. But no, you're absolutely right.

DR. SAXON: Jay, I have a problem with this experiment, and that is mice are so different than people in their LPS response, it seems inappropriate. Mice have a specific receptor for LPS that turns on IgE, that turns on IgG1. People don't have it. There is no relationship to the murine system and the human system in this regard. There is no relationship. LPS is a specific mitogen in mice. It does the B-cells. It does not work in humans. They're totally different systems. So this is very interesting for mice. It has no reflection, unfortunately, on what happens in humans.

I think the experiment needs to be done, but I don't think this system addresses that question the

Europeans have raised about does LPS affect asthma. I think there is a literature, but the rich literature on LPS from Cliff Snapper and Fred Finkelman is because there's a specific B-cell receptor on murine B-cells that doesn't exist on human B-cells.

DR. SLATER: Actually --

DR. SAXON: That's true.

DR. SLATER: It is true. It is true that in vitro there are specific B-cell effects that you can demonstrate with LPS on mice. That is true.

DR. SAXON: Murine B-cells that do not occur with human B-cells in vivo or in vitro that I know of.

DR. SLATER: But the old studies that were shown of the effect of LPS on mice in vivo showed that you don't see this effect in T-cell-depleted mice.

DR. SAXON: Well, no question, you require a T-cell as well, but the LPS works on murine B-cells directly. The best way to make IgE in a mouse is LPS and IL-4, and it simply doesn't work in humans.

I think it's a great experiment, but unfortunately I think we'll have to do the experiment with a human. And can you give LPS to a human? Probably. Our guts are full of it, right? But we can't use this antigen because we wouldn't be allowed to make people -- we've sensitized people to KLH in another setting, and made

primary IgE responses to KLH in people. I think the way you have to answer it, which is a compelling question you ask -- I mean, I understand the literature -- is but the murine system I don't think will answer the homologue in the human being, unfortunately.

DR. SLATER: Well, I think you're right, but I think that the further implications of this, if in fact nasal and -- there are a number of different ways to approach this. One is to design a human protocol in which we'll give LPS to humans and see if it affects their IgE responses. That certainly is one possibility.

Another possibility I think is to try to dissect this out a little bit further. Again, with strain specificities of different mice.

DR. SAXON: I think the only mouse that's LPS-nonresponsive is the XID. Isn't it? And it makes IgE responses, though, so the XID mouse might, but most mice -- you'll have to get a good mouse geneticist.

DR. SLATER: There's actually a high variability of LPS responsiveness in mice.

DR. SAXON: Okay.

DR. SLATER: Again, I think the questions you're raising are certainly valid. The purpose of this wasn't to say this is how the human responses occur, but the specificity of mouse B-cells to LPS is an in vitro

observation that may not correlate to the actual mechanism of this effect.

In other words, to get the B-cell proliferation that you get, it is true that in vitro mouse B-cells are uniquely susceptible to LPS' effects. That's true. But in vivo, there's good evidence to believe that mechanism of this may not be related to the effect on B-cells of the mice.

This is actually a discussion that Donald Leung and I had at great length when this paper was being submitted to JACI. I think I convinced him. Maybe we can sit down and try to --

DR. SAXON: I wasn't the reviewer.

DR. SLATER: That's okay. Other people have made this observation, too, but I think it's good point, but I convinced him, and maybe afterwards I can convince you, too.

DR. SAXON: I'll do the experiment with you. We'll do it in a human.

DR. SLATER: Okay.

DR. SAXON: You give me the LPS, we'll set it up, and we'll do it with KLH in people.

DR. SLATER: We really see almost the same thing with IgG1 responses and IgG2a responses as well. In other words, a significant augmentation of the specific

responses to the fusion protein in mice that received LPS as well.

So when LPS is co-administered with Hev b 5/MBP, you get accentuation of the anti-Hev b 5/MBP IgE and IgG responses, and you also, and I'm not showing this, get specific anti-Hev b 5 and anti-MBP spleen cell proliferation responses as well.

So why are we interested in lipopolysaccharide for our lab? Well, as we've already sort of alluded to, it's something that we really need to know about if in vivo it really does have effects. First of all, in terms of the mice, we'd like to see whether there's a functional correlate to these antibody findings that we see. We actually have set up the Buxco mouse plethysmograph, and we're going to be doing some studies in these mice to see whether these mice actually have increased sensitivity to antigen in terms of a functional reactivity. We want to know whether the amount of LPS in latex glove powder is significant. We want to know whether these effects are strain-specific or antigen-specific.

Finally, and again getting right back home to what this lab spends its time worrying about, we want to know whether the amount of LPS in allergen extracts matters, whether quantitatively it's something that we need to be concerned about as we move forward into the future.

Again, just to summarize the research program, we're trying to construct a program that's relative in terms of our regulatory function, in which we look at glycosylation issues, enzyme activity issues, identification methods that are improved, more accurate, and can tell us things about the glycosylation state of the proteins that we're interested in.

Further, we're interested in exploring further some of the newer methods of immunomodulation that might be pursued over the next decade with increased knowledge of epitopes, DNA vaccines, and the possible effect of LPS on sensitization.

Thank you.

DR. OWNBY: Thank you, Dr. Slater.

Any further questions from the committee? Betty?

DR. WRAY: I was just going to make a comment that as we think about monoclonal antibodies, we have seen a patient with anaphylaxis to Neupogen, so we may be introducing new antigens at the same time we're giving the antigens we're trying to get in.

DR. SLATER: Right. Absolutely.

DR. OWNBY: We're making it very easy for the chair here. We can entertain a few other questions or we can move on and move into our -- we're scheduled for

another 15-minute break, and to be back at 10:50. Okay. Let's go ahead and take a break. We'll be back at 10:50, then.

(Recess.)

DR. OWNBY: I think we're ready to get started again. Our next speaker, our only speaker -- boy, Jay, I'm glad to see they hired you or we never would have had a meeting today.

(Laughter.)

DR. OWNBY: They're obviously getting their money's worth out of someone.

Dr. Slater is going to start with, I guess, the potency limits and some regulatory proposals.

DR. SLATER: Yes. We're going to be talking about some regulatory proposals, and when I talk about regulatory proposals, these are really a discussion of what our lab does in terms of the regulation of these products.

Committee members, I just want to call your attention to the prepackage that you were sent a couple of weeks ago. There's a draft memo that starts "Potency limits for allergen extracts," and in addition, relevant to that is a four-page handout that has appendices on top, and these are appendices for that memo. We'll be referring to that in a few minutes.

First of all, before starting, I want to

acknowledge the role that Rich Pastor, the head of the Laboratory of Biophysics, played in the development of some of these ideas. Those of you that know Rich will sort of see his hand in a lot of this discussion. Clearly, a lot of these issues are issues that I never even thought about much before August 31st when I came here, and so I really have needed much help in terms of guiding me through this, and I want to thank Rich for that.

The assays that we currently use to assess standardized allergens are either radial immunodiffusion assays for cat extracts and for short ragweed extracts or the competitive ELISA for the mites and the grasses. I'm not going to be discussing anything that has to do with the RID assays at this time. We're going to be focusing just on the competitive ELISA.

One of the features of the current model of the way we assay these products that was a little bit bothersome to me is that the release limits that we set -- in other words, the acceptable range for a product that is sent to us for evaluation -- is really driven by the technique that we use.

So if you go back to skin testing, the range of precision of the assay if it's wheal or erythema could be as much as three and a half-fold up to 13-fold, with the erythema with its steeper dose-response curve having

significantly greater precision than using wheal alone.

RAST inhibition, based on the standard deviations of that assay, the limits were set at about .46 to 2.12. This is using 1 as the desirable mid-point, and of course it's only a mid-point if you look at this in log space and not in linear space, but 1 is the desirable mid-point in our limits that we set with the RAST inhibition for .46 to 2.12, or about a 4.6-fold range from bottom to top.

Finally, when ELISA inhibition was used, or competitive ELISA was used, that was somewhat more precise and if you did three replicates -- in other words, three separate assays -- and pooled those results, you could have results that were somewhere in the order of .7 to 1.43, or significantly improved at a two-fold range of equivalence.

However, theoretically at least, if you continue to drive your limits by the technique, you're getting more and more and more precise in terms of your measurement technique, and it's not clear that biologically this greater precision really buys you much in terms of having a better product. So one of the things that we wanted to look at was what information there is from the literature about how much precision you actually need on allergen extracts the way they're currently used.

In addition, a problem with the current

paradigm is that we have identical limits set for industry and for CBER. Well, that shouldn't strike anyone as a problem offhand, except that it is technically a problem in that as the manufacturers send us products that are closer to the limits, their chance of failing a product that really may fall within these limits is significant. If we have limits set at .7 to 1.4 and they send us a product that really has a relative potency of .75, that curve around .75 gives them a significant failure rate.

So while there's nothing conceptually wrong with this idea, there's a significant practical problem, and that is that we will really severely penalize a manufacturer that sends us a product within the limits, but one that is close enough to fail.

DR. SAXON: Jay, what happens if you had a sample that had a wheal, say, of .27 or 3.65 and you moved it to the ELISA? Would it still generally fall within -- I'm trying to get an idea. You said, for example, the wheal has the biggest, I guess, limits.

DR. SLATER: Right.

DR. SAXON: Would samples that are passed by the wheal criteria also pass by the ELISA inhibition or would many of those fall outside it? Do you know?

DR. SLATER: You know, I don't know the answer for sure. I don't think that they would. I think that

they would fall outside.

DR. SAXON: That's what I was trying to find out. So the test is -- okay.

DR. SLATER: So the test really is narrowing the product around --

DR. SAXON: That's what I meant, as opposed to.

DR. SLATER: And again, there's nothing inherently wrong with that, but I think it does raise questions that I think we need to try to look at in a critical manner, and one of the things that we started to do was to really start to look at the allergy literature and see what we knew about what an acceptable range would be.

Now, you can divide this up any way you want. The way I thought was reasonable, and the way I described it in the memo, is looking at these products from a therapeutic point of view, from a diagnostic point of view, and from a safety point of view. It seems to me that these are the three spheres of interest that we should have regarding these products.

As luck would have it, two out of three of these were kind of easy to come up with conclusions, but the third one was hard. So I'm going to dispatch with the two that were easy pretty fast, unless there's some objection, and probably spend an inordinate amount of time

about the one that was hard, but I think it's important to plug through the process.

If you look at studies on therapeutic immunotherapy, it's quite clear from most of the studies that there's approximately a 10-fold range of therapeutic equivalence. That is, once you get up above a certain level, if you look at sort of the mean doses that are used in most of these studies, you can go up and down several-fold for a total therapeutic range of about 10-fold without losing any therapeutic efficacy.

Again, remember what we're looking at here and what happens if you broaden your limits and you go from one product to the next, and because of those broadened limits you have somewhat increased differences between the products, and there's good reason to believe from the literature, and I cite the references in the memo, that up to a 10-fold difference really will make no therapeutic difference, assuming you've gotten into the therapeutic range to begin with.

Likewise, for diagnostic testing, for the actual precision of skin testing, again, it depends on whether you use wheal or erythema, but for erythema there seems to be a three- to four-fold range at which there was more or less diagnostic equivalence of allergen extracts. For wheal, it was somewhat higher, perhaps as much as

eight- to 10-fold range. Again, those references are shown in the memos that you have, and again, these data are pretty straightforward. There's really some fairly good studies that show that.

It was with safety -- and here you have the simple designation of a four-fold range for safety. This actually was the product of some fairly difficult twisting and turning with the data, and the reason is that it was hard to find good studies that addressed safety issues for allergen immunotherapy. There were a limited number of studies to begin with. Many of the studies had small numbers of subjects in them. Only a few of the studies really looked at highly allergic subjects which, after all, is the population that we really are interested in looking at. Fewer still did these studies with well-standardized and characterized antigens, and fewer still used consistently-defined endpoints for the studies. So with the safety data, I will admit to you right from the outset, we were in the position of trying to extract information from a relatively small database.

That being said, I am committed to the idea that this is something that we should try to do, because I think it's important to try to develop a paradigm for analyzing these data and looking at this information. Hopefully, there will be more information coming down the

line as we go along, and I think it's important to try and plug through the methods for how you would analyze these papers, compare them, and come up with some increased reaction rates.

So what we attempted to do is we attempted to find papers that fulfilled at least some of these criteria on the previous slide and identify adverse reaction rates at therapeutic doses. We were interested in what the adverse reaction rates were at doses that worked in terms of immunotherapy. We wanted to determine the increase in adverse reaction rates with increased doses. In other words, what was the relative difference, what was the slope, of the increased reaction rate with log dose increases? We used semi-log plots simply because you got better linear fits and it was easier to extrapolate when you could do that.

We separated analysis of per injection data and per patient data. Several of the papers that we used had both per injection and per patient data, and we could argue about which are more relevant. My visualization of this issue is you have an old bottle that you can't use anymore, you've purchased a new bottle, and it's the reaction at that next dose that you're concerned about, so I was actually more interested in the per injection data, but you could arguably say the per patient was important as well.

We in fact looked at both.

Then we attempted to pool these data by either averaging them or by doing weighted averaging, and we also attempted a logistic analysis, which I'm going to show you as well.

So this is just samples of some of the curves that you come up with when you look at these papers. These are semi-log plots. This is the log of the dose at the bottom. These are reaction rates in percentages on the abscissa. These are actually data extracted from a single paper by Haugaard using dust mite allergens.

As you can see here, in this one, for instance, the slope was about 4.16. This group of patients, the slope was somewhat higher.

DR. SAXON: Can you explain that a little better, Jay? I'm a little lost.

DR. SLATER: Absolutely.

DR. SAXON: So what you've got on the bottom is a log of the dose.

DR. SLATER: It's the log of the dose that was given, and we're plotting the reaction rate in percentage of injections. This is per injection.

DR. SAXON: And as the doses were increased, so what you're saying is as you increase the doses in this logarithmic fashion, this is the percent of reactivity.

DR. SLATER: Right.

So just to be even more concrete about this, what you see here, the physical meaning of this slope is that as you increase one log unit or 10-fold, you have a 4 percent roughly increase in reaction rate if you can believe this linear regression, which has a decent R squared, but not terrific. This curve would suggest about a 9 percent increase in systemic reaction rates with a 10-fold increase in dose.

PARTICIPANT: What's the difference between the two charts?

DR. SLATER: Well, this was all of their patients, unstratified, and this was their patients who were at maintenance dosing alone. Okay? So I wanted to analyze both of those.

There was another paper authored by Paul Turkeltaub. This one was using Amb a 1. This was with a somewhat larger group of patients, and the two groups of patients here, this is all of his patients, and again you can see here that the R squared is rather poor from this, but again, roughly we get about an 11 percent increase in reaction rate with a 10-fold increase in dose.

This group of patients is of special concern. These are the patients that had reactions that required epinephrine, and in this situation we see approximately a

17 percent increase in reaction rate, again with a 10-fold increase in dose.

Dr. Saxon, you still look concerned.

DR. SAXON: No, I understand it.

DR. SLATER: Are you following this?

DR. SAXON: No, I'm following it.

DR. SLATER: Okay.

These are data from an unpublished paper from Scolase and John Younginger that was done with both mite and ragweed allergens. This is the mite data alone. Those are the relatively smaller number of patients. Only about 70 patients were in this study, but we were looking for any good data we could get, and so we took this as well. This was done with standardized allergens, and again, the slope in this one was a fairly good R squared. It was about 9.7.

So then you've got these data. The question is what do you do with them? Well, you can just average them, and there's really no satisfying answer for how to pool these data. There are problems with every way of analyzing them, but we tried several different ways.

If you pool the per patient data and average them, you get a slope of about 13.4. If you pool the per injection data, you get a slope of about 8.2. If you look at all of it, you get a slope of about 10.

Again, let's go back to what this means. It's

a 10 percent increase in adverse reaction rates with a 10-fold increase in allergen relative potency.

We also attempted weighted averaging, and the idea of weighted averaging was to use the data that seemed to be more precise or more accurate with higher weighting than the data that had the bigger spread. That actually didn't change the data all that much in the end. About 13.6 per patient, and it lowered the per injection to about 6 percent per 10-fold increase, and it lowered the all pooled data somewhat to about 9.3 percent, but there was not a huge difference between those two.

DR. UMETSU: Did you say that these are patients at maintenance?

DR. SLATER: Not all of them are at maintenance. The patients that were at maintenance were the second set of Haugaard patients, and those were the only the patients that were specifically stratified out, but when we analyzed the data we only looked at the doses that were within the therapeutic range in that study. So the authors may not have selected the patients that were at maintenance, but we only counted the points on the curve that were reported in the therapeutic range in that study.

So for instance, in Haugaard's other data, where he's pooling all the patients, we actually only looked at doses greater than .7 micrograms of Der p 1,

because in his paper he showed that only doses above that actually were therapeutic, and we excluded the lower doses.

DR. UMETSU: I guess the question is I don't see what you're leading to. People who are allergic, if you give them a lot more antigen, they usually are going to react. So where are we going with this data?

DR. SLATER: Well, where we're going with it is an effort to try to estimate what kind of precision we need for the allergen extracts from the point of view of safety, and the question is can a patient who's allergic who's on immunotherapy tolerate only a two-fold increase if that increase happens as a result of lot switching? Can they tolerate something more, a three-fold or a four-fold?

Again, I admitted at the beginning that this is trying to squeeze the information out. There are some reassuring things later in the analysis that I'm going to show you, but I wanted to plug through this initially.

The logistic analysis is based on an analysis not of just P, but of P over 1 minus P, where P is the probability of a reaction rate at a given dose X. The effort here is when you're doing a distribution, the distribution of P, is that P only goes between zero and 1. It could be between zero percent and 100 percent. In doing this ratio of P over 1 minus P, it gives you a ratio from zero all the way up to infinity, which gives you a better

distribution that you can analyze using this kind of analysis. So the log of P over 1 minus P equals the slope times the log of X plus B.

Unfortunately, when you do this analysis, you can't really plot out a line at any point, and the variation, the relationship between P and the log of X varies along different Ps, and you have to treat all the data separately. There's really no way to pool the data at all.

But if you accept that a range that yields a 5 percent increase in reactions at geometric mean doses is somewhat acceptable, and this is gone through more in that appendix that I handed out, and you look at the different parts of the different studies that we were able to look at, you can see that in Haugaard's overall data about a 4.6-fold increase led to a 5 percent increase. In his patients at maintenance, they tolerated only about a 2.4-fold increase, giving a 5 percent reaction rate.

Turkeltaub's data, a five-fold increase gave a 5 percent increase, and then finally, if you take Turkeltaub's subsector that required epinephrine, again about a 1.7 to two-fold increase led to a 5 percent increase in reaction rates.

So then we come to the question of how tightly should we actually be regulating these allergens. The

question really has to do in the end with what is the sigma or standard deviation of the products that are actually sent to us? How does it compare to the sigma of the assay?

Just thinking about it conceptually, if in fact the sigma of the products is small and they're sending us products that are fairly tight as a consequence of the manufacturing techniques, the quality control that goes on before they send products to us, if they send us products that are pretty tight around a relative potency of 1, and our assay is pretty broad in terms of its relative potency, well, then we're really not so concerned about the average product. We just want to make sure that the outliers are cut out. We want to make sure that we lose those outliers.

So we want to look at the relationship between the sigma of the products that we are sent and the sigma of our ability to look at those products and, assuming a Gaussian distribution, the sigma of the observed products equals the sum of the sigma of CBER's assay and of the manufacturers' products, and what we're looking for is this.

Well, how do we look for that? Well, we can make an estimate of what our observed sigma is. If you look at from 1995 to 1997, we rejected 53 out of 414 products that were sent to us in 1995 to 1997, or about 13 percent of the extracts failed. That translates into an

observed sigma of the products plus our testing of 0.12.

Of all the slides, this is the only one I couldn't get to work on this screen, so I'm going to switch over to a smaller screen.

The sigma of our testing is .1375 divided by the square root of 3, or about .08. The sigma of the manufacturers, then, is the square root of the difference between these two squares, or, in other words, the sigma of the manufacturers is .092.

Notice that our sigma is about .08. Theirs is about .09. That's not too bad. They're actually pretty close to each other. In fact, that translates into a fairly tight sigma of the products that were sent to us during this period in which 13 percent of products failed.

So again, if the sigma of the products that are sent to us is high -- in other words, if they're sending us a very broad spread of materials -- then we need to really insist on equivalence to 1 at an acceptable alpha. In other words, we need impose our curve on top of theirs and, in effect, narrow it.

On the other hand, if the sigma of the product that's sent to us is low, then we really need to test at the boundaries and make sure that we're not being sent any outliers, but for the most part we don't have to worry about the products that we're being sent.

Another question that comes up is what is the likelihood of lot differences? What we really want to know is what's the likelihood that if we get Product A and it expires, and we switch to Product B, that we're going to have a big-league difference between A and B that our patient is going to see and get into trouble with.

Well, it turns out for a Gaussian distribution, and this is also described in the appendices for your reading on the way home, that the ratio of Product B to Product A can be analyzed in one of two ways. You can either look at the average ratio of all sequential products within the distribution or you can look at the level below which 95 percent of the ratios fall.

It turns out that for a Gaussian distribution the mean R is about 0.8 times the sigma and the 95 percent maximum R is about 2.77 times sigma, and you'll notice that I haven't talked about the actual limits anywhere here. This is entirely a function of the shape of the curve and of the breadth of the curve that they send us. So when sigma is .092, the mean R or the average ratio of two sequential products ends up being 1.18.

What does that mean? That means that on average, given the sigma that we're seeing, two products sent sequentially are going to differ by 18 percent in relative potency. Not two-fold, not three-fold, not 4.6-

fold, but 18 percent.

Okay, that's the average, but what's the real maximum? The real maximum we know is going to be set by our limits. If we set three-fold limits, the maximum's going to be three. If we set four-fold limits, it'll be four. Whatever the real maximum is, we can set that.

But what's the maximum given the sigma of the curve? The R 95 percent, it turns out, is 1.8. So 95 percent of sequential products that are sent to us based on the best data that we have available will have an 80 percent or less difference between two sequential products.

One very important point that I'm sure many of you thought of, but I need to make explicit. These sigmas are aggregates for the industry. It's not necessarily true that each manufacturer has the same sigma. In fact, it would be hard to imagine that they all have the same sigma. Remember, the sigma of the products that are sent to us is an aggregate of their manufacturing techniques, their internal quality controls, and what kind of screening that they do. So whereas this is very reassuring in terms of the aggregate, it's not necessarily the case that each manufacturer is going to have the same sigma.

But what I'm leading to is this. What we would like to propose is that we set the CBER limits, based on the literature that's available and based on this analysis,

at between 0.5 and 2.0. But we are going to recommend that the manufacturers' limits, which are established in their PLAs at this point anyway, remain unchanged. If they do three replicate products, then their range is going to be .7 to 1.43. If they do six replicates, which many of them are doing, incidentally, then their range is going to be even tighter, .78 to 1.29.

We will not accept products that have been tested by them to be outside of those limits, but we will not fail products unless when we test them they fall outside of these limits.

Now, why have two separate limits? Again, this gets back to the problem that I addressed in the very first slide. Let's say we set the limits at .5 to 2.0. Let's just say that was an acceptable range and that's what it's going to be for us and for the manufacturers.

Here we have the same limits set for us and for the manufacturers. If the manufacturer sends us a product with an RP of 1, then none of us has any problems with this. It'll work just fine. Nobody's going to fail or very few are going to fail.

But let's say we told people that an RP of .5 or of 2 is perfectly acceptable, and they send us a product that really is .55 or 1.95. Well, they've got a substantial chance of failing at that rate, as much as 50

percent if they get close to the limits.

However, in this somewhat more complicated slide, if we set the CBER limits at .5 to 2, but we continue to have the manufacturers' limits narrower at about .7 to 1.4, what happens when they send us something at the borders of what we've told them to send us? Well, if they send us something that's .7, here's the standard curve around that, and these are the number of products that we're going to fail at a limit of .5. Likewise, if they send us something on the high end, these are the products that we're actually going to fail on the high end.

In fact, you can quantify that. You can see that if the manufacturer performs six replicates and they use these tighter limits that many of them are already using, the chance of failure is only two and a half percent, very, very small. If they decide to do only three replicates and they set the limits between .7 and 1.43, the chance of failure remains substantial, 10 percent, but that's not as bad as it would be otherwise if we had the same limits that they did.

DR. SAXON: Jay, also a good thing about what you've done, then, also is because if the manufacturers had your limits, they're going to pass lots that are actually .46, and you may pass them, too. Then they become dangerous. Maybe not dangerous, but they may be very low

or very high, because of the shape of the curve.

DR. SLATER: Right, and the other concern obviously is they're pushing the limits of what we --

DR. SAXON: Right, but you may also not catch it, because of the shape of your curve at the same point.

DR. SLATER: Exactly. Absolutely.

So I think this is a reasonable limit based on what we know, and just to summarize what we're suggesting, these are the current limits and these are the proposed limits. The current limits are that the acceptable RP is either .7 to 1.4 if N equals 3 or .78 to 1.29 if N equals 6. It's the same for manufacturers and for CBER, and again, it's largely technique-driven. With this paradigm, we get a better technique. We'll get tighter control and tighter control.

The proposed limits, leaving the manufacturers exactly as they currently are now, but broadening our limits of failure to 0.5 to 2. As limited as the technique is, we think this is study-driven, and we'd like the limits that are set to be based more on the biology of what's happening with these extracts when they're given to people than on the techniques themselves.

Another point to point out, and that is that nominally these limits look like the European standards of .5 to 2.0, but you have to realize that there's a not so

subtle difference between those, and that is that we really have specified the statistical limits that we're going to accept. They have set .5 to 2.0, but really haven't specified the kind of confidence that is required for that. So this I think does exert greater control, although I think it's a rational degree of control and more justifiable based on the literature.

The next aspect of our activities in LIB that I would like to propose some possible changes in is our dealing with protein measurements in allergen extracts.

Now, the current standard for measuring protein in standardized allergen extracts is to use the modified ninhydrin assay that was designed by Paul Richman, and the purpose of this assay was to develop a good, reliable assay for the protein content in allergen extracts. Clearly, this is the most reliable method for doing so. There's absolutely no doubt about that.

However, there are some odd things about the standards that we apply. One is that there really is no acceptable standard range for these protein contents. They are, rather, informational, and in order to pass based on the protein content, the CBER value must fall within 40 percent of the manufacturer's value.

So in other words, unlike the relative potency, where we have a standard number that the manufacturer has

to strive for, rather what we do is we are collecting this information, we do collect the information, and the CBER value has to fall within 40 percent of the manufacturer's value.

What is the ninhydrin assay? The protein is hydrolyzed under alkaline conditions, it's cooled and neutralized, and then ninhydrin is added. Ninhydrin leads to the oxidative deamination of primary amino acids that are released during this hydrolysis process. The hydrindantin is released with the corresponding aldehyde and some free ammonia. The free ammonia reacts with another molecule of ninhydrin and this colored, oxidized product is formed that is red spectrophotometrically.

Why do we recommend keeping a protein standard? Well, it's certainly an assessment of purity. It's a way of assessing the purity of the product that you're being sent, it will alert you to the presence of foreign antigens, it can serve as an internal quality control, and as an estimate of protein content for other assays. It's also a way to control for the possible effect of protein content on other assays.

What are the problems with the ninhydrin assay? Well, it's cumbersome. Certainly, compared to the other protein assays that are out there, it's definitely more difficult to use.

There's another theoretical problem that's been raised, but never proved, and that is it may be too accurate. It really is as close as we can get to an amino acid analysis without doing an amino acid analysis, and as such, it probably picks up small peptides and amino acids that are of no immunologic significance at all. So we may actually be detecting substances that are not significant as components of the process.

DR. KING: Let me just be clear exactly what you said on the last point. You're picking up amino acids and peptides, so if the extract is a dialyzed, you're going to get a different ninhydrin value.

DR. SLATER: Theoretically, that's the case.

DR. KING: It's not theoretical. It's really true. That's definitely the case. It just seems to me -- I mean, I agree with you it has certain advantages. It's, again, another relative assay if you don't take care of the dialyzable portion of the low molecular weight components.

DR. SLATER: Thank you.

What are the problems with the other methods? Well, the problems with the other methods are well known. Glycerol, which certainly is an important part of all the products that we're concerned about, can affect these assays profoundly. Other chemicals, such as phenol, can affect the assays.

The most important, disturbing problem with the other assays is they all have requirements for the presence of specific amino acids. Cysteine, tryptophan, or tyrosine are required in order for these assays to work, and in particular, a protein that's close to my heart, Hev b 5, lacks all three of these residues and is essentially invisible to Coomassie blue on assay. So there are definitely problems with the more readily available and easier to perform assays.

We were concerned about the possibility that the protein content of an extract might be important in terms of the accuracy of measuring the relative potency of that extract using the competitive ELISA. So we wanted to see whether adding BSA, spiking an extract with BSA, affected the accuracy of the relative potency assay. We added BSA up to 125 micrograms per mL in one of the two steps that we thought might be relevant. One was the coating step and the other was the inhibition step. The antigen was D. pteronyssinus.

Now, before we even start, you would predict that there would be a profound effect if you add BSA in the coating step, and that was in a sense our positive control. We would hope that we would not get an effect with the inhibition step, but that's the part that we were actually most interested in.

So here you see the predicted effect, what we have predicted effect, of adding extraneous protein to the antigen coat in the competitive ELISA. What you basically see here is a family of curves. As you're going down these curves, you're going to increasing concentrations of protein that were added to the D. pteronyssinus extract during the coating step. Not surprisingly, you have much less protein binding, much less specific protein binding, to the wells, and you have inhibition of the optical densities that you see.

Since this is a competitive ELISA, this decreased optical density translates into an increased relative potency, and look at the scale of this relative potency. This is not a 1.0. This is a 10, 20, and a 30. So you see here that when the amount of BSA that you add is low -- say, less than 9 or 10 micrograms per mL -- you have relative potencies that are just about where you would expect them to be. But then when you get up to about 15 micrograms per mL, you have the rather ludicrous result that your extract looks like and has a relative potency of 25, certainly not a very acceptable situation with the assay.

That's the bad news. On the other hand, the good is that with the inhibition step, there seems to be almost no effect of extraneous protein, which, again, you

would have predicted, but it's reassuring to see it experimentally, especially in the context of demonstrating a profound effect on the coating step. These are the family of curves of the inhibition step when the protein is added in the inhibition step, and really, statistically they're indistinguishable.

So what does that actually mean? It means that for the purposes of the competitive ELISA, the protein content of the products that we are sent for analysis doesn't matter, because the products that we are sent for analysis only are used in the inhibition step.

However, what do we coat with? We coat with products that we have selected from commercial products to use as our reference standard. Therefore, the burden is on us to make sure that the protein content of the reference standards that we choose is relatively constant and is not wildly changing.

So our conclusion from this is that the protein content probably doesn't matter all that much in the products that are sent to us, except we need to be aware of this as a problem in terms of our choice of a reference standard.

This is a slide I showed you a few minutes ago. Why should we keep the protein standard? Let's go down these one at a time. Purity assessment. It is a good way

to tell how pure a product is. However, it's not clear that this matters all that much. It's not obvious whether we would want to have a lower or a higher protein content. All things being equal, I think we would decide we'd probably want to have a lower protein content and a purer product, but that's not obvious in all situations.

Furthermore, we have other ways of assessing purity that I think are probably more accurate, such as the blotting, to look for extraneous bands that we need to worry about, and perhaps working on other identification methods, such as the MALDI-TOF, to look for extraneous proteins.

In terms of internal quality control, I think with the antigen-specific tests that we have for these allergens, the internal quality control probably could be completely fulfilled by the antigen-specific tests.

As an estimate of protein content for other assays, it's very likely that other protein methods can be used to do that.

For the possible effect of protein content on an assay, I think we already discussed that that probably is not much of an issue.

So after thinking about this, there were several obvious choices, which are detailed in the draft memo that you have, but I think the most conservative

approach would be to continue to require an informational protein assay that CBER will use to assure the consistency of reference standards, but manufacturers will, if this is approved, be able to choose any established, validated protein assay to monitor their products from time to time.

In other words, if a manufacturer wishes to switch from the ninhydrin to another assay, they will need to validate it, they will need to show that they were able to detect protein differences by using it, but we will be open to the suggestion of an alternative protein assay that can be used.

We will no longer routinely assay the protein content or reject samples based on the protein assay results.

Note, incidentally, just to reiterate what I said a few minutes ago, that we're not really abandoning a standard. There really wasn't a particular standard before, except to impose the informational assay and to make sure that the manufacturer's test correlated with our test.

The advantages of this approach are that data will be, within a given manufacturer, internally comparable, but data will not be comparable among different manufacturers if they all choose different tests.

LIB will not replicate the data as part of

routine lot release, and as a result, there's no possibility of lot failure based on the assay.

I just want to clarify a couple of things. The recommendation applies only to standardized mite and grass allergens. Standardized hymenoptera venoms will continue to be assayed by the ninhydrin assay as currently required, and the results of protein assays performed on standardized mite and grass allergens may not be used in product labeling materials.

I'm at the end of my portion of today's proceedings. I just want to summarize what our regulatory goals are. We want to continue staff stability and expansion. We want active improvement of the support program for standardization of allergens. We want to support future standardization efforts, per advisory committee recommendations, and we've talked about our goals in terms of research activities.

I just want to close with one final comment. I know this is an ambitious program that I've talked about today. I must tell you that, since coming to FDA in the end of August, I have been struck at what an enviable situation I am in. I have had terrific colleagues who have really been incredibly helpful, highly professional, very, very good support. I am in the remarkable position of having terrific people above me and terrific people under

me.

I wouldn't have put forth this program if I didn't think we would be able to actually be productive, and, believe it or not, I think by the time we meet next I'll probably have more to say.

Thank you very much.

(Applause.)

DR. OWNBY: That's only going to be in another four years.

The committee have any other questions for Jay at this time? Dale?

DR. UMETSU: Can you just review exactly how the grass and dust mite are standardized? What assays are you looking at?

DR. SLATER: The major assay that's used is the competitive ELISA assay. In that assay, we coat plates with our reference standard, and then incubate with the serum pool that we have, either without any competing allergen that's added or with different doses of the competing allergen that's added.

We compare the results that are obtained with the product that's sent to us by the manufacturer with the results that we obtain using our reference standard for that allergen, and then based on the location of the parallel curves, we determine a relative potency of the

product compared to an RP of 1 that's set by our product.

DR. UMETSU: And where does the reference antibody come from and also where do the reference antigens come from?

DR. SLATER: The reference antigens come from the manufacturers. In other words, we choose a reference standard when the previous reference standard expires.

The idea of the reference replacement program that I described about two hours ago was to make that reference replacement program proactive, so that we would actually select the new standard, compare it to the old standard, and make sure that it was comparable. We would distribute it to the manufacturers for them to test, get feedback from them, and then finally choose that as the standard.

Likewise, with the reference antisera that we use, we would pool them from immune sera that we purchase, test it, and send it out to manufacturers for testing.

It's really what I was talking about at the beginning, that it's a sort of a long, iterative process of going back and forth. It gets considerably shortened when we run out of the previous one and have to move fast to a new one.

DR. OWNBY: Anything else?

(No response.)

DR. OWNBY: The next item that was listed on the agenda we're going to --

MS. MONZER: I'm sorry. I have just one question. I'm Martha Monzer, FDA.

For the protein assay, what about standardized cat and ragweed? You didn't mention those on your last slide.

DR. SLATER: Right. Standardized cat and ragweed, we were not going to do that. First of all, it's because we use the RID assay for that, and we were a little reluctant to change what we were doing at this point. We really focused on the grass and the mites.

DR. OWNBY: Thank you very much, Jay.

The next item that was on our agenda we're going to postpone for later this afternoon. We are waiting for Dr. Storms, who's going to make that presentation, and we'll hopefully be able to come back to it in the open public hearing sometime around 2 o'clock.

The next status report is the Class IIIA allergen extracts, and I believe Steven Falter from the Regulations and Policy Staff is going to present that.

MR. FALTER: Good morning. As you can see from my title, I come not as an expert on allergen extracts, but as sort of the baron of the bureaucracy at CBER, and as one of the last of the Mohicans that has the knowledge of the

classification review process.

Because we have ongoing efforts to complete this classification process, I wanted to update particularly the committee. They have been asked, they have been consulted with in the past a couple of times on individual issues, and as we carry through the process, I'm sure that we'll be back asking multiple questions. We have no specific time line as to when we'll be consulting the committee on scientific issues, but I'm sure, at minimum, once we issue a proposal there will be plenty of public comment in which we may both want a public forum for public discussion and want the recommendations of the advisory committee on individual issues.

So on the next transparency, to bring you long up to date, it all began when we moved our predecessor, at least, from NIH to FDA as the Bureau of Biologics, when at the same time the drug folks who were already at FDA were undertaking a thorough review of their older products to reevaluate their safety and effectiveness.

Now, for a slightly different legal reason, we decided to also review biologics, mainly because a number of these products, including allergenic extracts, were quite old, and the standard for safety and effectiveness at the time of approval might be quite different than what has evolved through time. So we began this process through a

codified process, and allergenic extracts was one of six different product groups that were reviewed under the biologics umbrella.

In '81, we received the final report of the Panel on Review of Allergenic Extracts containing their recommendations regarding the safety and effectiveness of all our known allergenic extract products. Then it became FDA's turn where, very slowly and deliberately, we first issued a proposed rule in 1985, and then finally, in 1994, we issued a final order in which a number of allergenic extracts -- not many of them very important, but the actual numerical count was quite large -- the licenses were revoked for those products.

Now, when we began the process, that should have completed what our obligations were, but a glitch developed in the system. On the next transparency, it identifies that. One of the options for both the advisory committee and for FDA was to put a product -- and not just talking about allergenics specifically, but any of the biologics reviewed -- in a regulatory category called Category IIIA.

Now, what the game plan would have been for that sort of product was that the finding was there were insufficient data to determine whether the product license should be revoked or retained -- in other words, there are

questions of safety and efficacy -- but based on a risk/benefit analysis, usually both the advisory committee and FDA decided they should remain on the market pending completion of further testing.

Well, this became a problem in the mid-80s. One, the legality of this category was challenged on whether we could allow the continued marketing of a product for which there are questions of safety and efficacy, and two, when you speak specifically of allergenic extracts, it would be a considerable undertaking if we were to mandate further clinical study of each and every allergenic extract through a very set, monitored process, just simply because the number of extracts that would have to be dealt with. Certainly, that still would be going on for quite some time and may not be feasible.

On the next transparency, what we decided to do is revisit, that despite the fact that the evidence may be lacking, we should, as best as we can, make a decision regarding the safety and effectiveness and labeling of these products. Two options were offered. The very obvious ones of Category I would be safe and effective, Category II, unsafe and effective or misbranded.

Actually, there's a little subcategory for a product that is absolutely necessary to stay on the market, even though there are questions regarding the safety and

effectiveness. None of the committees nor FDA has yet to have used that category.

Now, to interpret that specifically for allergenic extracts, they were all reviewed on both their diagnostic and therapeutic uses. In other words, one product may have actually two different findings, one for its diagnosis and another for its therapeutic use. An example would be safe and effective for diagnosis, unsafe and ineffective for therapeutic use.

On the next transparency, what would be the end product? After we're done doing our dirty deed, just what would the result be? Obviously, for Category I, it would mean that the product was found safe and effective, and no action needed. If you had the dual categorization, then one option would be required revision of labelings of the product was indicated only for diagnostic use. Finally, if found lacking for both diagnosis and therapy, we would undertake the revocation of the license. Actually, it's not exactly that, but in effect it's a revocation of the license.

So what has happened is that we have received the recommendations of the Panel on Review of Allergenic Extracts, the comprehensive review of all those products put into IIIA, which, by the way, encompassed all those that were not put into IIIB, so it's pretty much all

existent allergenic extracts, I believe, unless there have been brand new ones approved since that time. We consulted on individual issues with the Allergenic Products Advisory Committee.

Now, we're preparing the proposed order, which would propose what we intend to do and offer an opportunity for public comment and further submission by the manufacturers. I can't avow when that would issue, but we have time lined it for within this year, by the summer of this year.

Unfortunately, that only begins the process. We have to issue then a final order, which would issue our final findings, mandate any labeling changes necessary, would pronounce those that we find safe and effective, and even then we're not done for those where we're going to revoke the license. We'd have to issue a notice of opportunity for hearing where, once again, further information might be submitted.

On the next transparency, if there are requests for hearings, we don't necessarily have to accept that request. There are criteria for whether we accept it or not, but there could be hearings, and then the final action would be a notice revoking licenses for those products that were found unsafe and ineffective.

This process, along with the processes for a

number of other groups of biological products, has been ongoing for a tremendous amount of time. It is by full intent of those who created the procedures for issuing such documents that there are long and tedious -- that is the intent, in that they want to make sure that the executive branch of the government is very deliberative and it prioritizes down to the nth degree as to what they intend to accomplish through this process.

Biologics through history since 1980 issues about two and a half documents of this sort per year, and recall that there are two primary documents that have to be issued, the proposed order and the final order. So that has been unchanged, although we're accelerating just a bit now in the numbers we issue.

It still remains something we're trying to accomplish, I think we're obligated to accomplish, and there are other products that we are reclassifying, blood and some vaccines, but allergenics is placed pretty close to the top, just because of the number of products being considered.

I've limited myself solely to process. I have carefully avoided any technical discussion at all, because I'm not the person to be discussing that. So that is my presentation. I don't know if there are questions.

DR. OWNBY: Any questions, then?

(No response.)

DR. OWNBY: Thank you.

When we were planning this meeting, one of the other issues that came up was a question on compliance issues and this panel, and I believe we have a presentation by Cathy Conn on some of these compliance issues.

MS. CONN: Good morning. I'd like to thank you for inviting me this morning. I'm going to discuss some common compliance and enforcement issues.

What I'd like to focus on is why the agency takes enforcement actions, the process that we go through, and also I've been asked to address on how some supply issues are taken into account when we deliberate the process of should we take an enforcement action or not.

Prior to taking an enforcement action, an inspection is generally conducted. In the past, those inspections were conducted by CBER personnel. Currently, they're being conducted by Team Biologics, and towards the end I'll talk a little bit more about Team Biologics.

I'm only going to discuss the more common enforcement actions. There are many more on the list, but they're not the most common.

Biological products are unique because they're regulated by two federal laws, the PHS Act and the FD&C Act. The actions taken against the license are under the

Public Health Service Act. Seizures and injunctions listed up here are sanctions under the Food, Drug, and Cosmetic Act. To date, there have been no seizures or injunctions of allergenic products, but it is in the realm of possibility, so I thought I'd better add it to the list.

A little bit more about warning letters. They are a notification to affirm that we find that there are deviations from, generally, the good manufacturing practices. Those are the regulations in 21 CFR 211 that tell a manufacturer how they're supposed to manufacturer a drug product. They can also be deficiencies from the 600s, the biological standards.

The warning letter is sent to the firm. We expect prompt corrective action to correct any of the problems we've identified. The effect that a warning letter may have on a product is that, legally, there's no prohibition to distribution. In reality, sometimes, though, firms may have to slow down production in order to get a compliance plan together to correct their problems. So production may slow down and, in effect, distribution may slow down a little.

Also as the result of warning letters, a firm may have to conduct recalls. We may have identified a problem with a particular lot number that's out on the market, and a firm may have to take steps to recall it.

The agency policy is usually to issue only one warning letter. Generally, after the first warning letter, we're starting to contemplate other actions that we may need to take.

If anyone is interested, warning letters are posted on the FDA Web site. I don't have the exact Web address for you, but if you go in there and you search around, you will find them. They are there.

Seizure is to remove product from the market. Generally, what happens is we've identified problems with particular lot numbers, and we go out and we seize that product, and we prevent it from being further distributed, and in some cases prevent it from being used. It can be an action taken against either licensed or unlicensed products.

An injunction is a court-sanctioned action. We go to the courts and say this firm has had uncorrected violations, we've told the firm about it several times, they've had warning letters, we've had meetings and whatever, and we feel that the court now needs to supervise the activities of a firm in order for it to come into compliance. The injunction can affect licensed and unlicensed product distribution. It can also affect the interstate and intrastate distribution.

When we write an injunction, oftentimes we'll

enter into what's called a consent decree, where the firm and FDA are negotiating, with the court's oversight, what is going to happen during the terms of the injunction.

In some cases, the problem could be bad enough that we need to stop distribution of the product. In other cases -- for example, if it's the only product available -- there could be times when the agency would say, well, we'll allow distribution of the product, but only under tightly controlled conditions, and generally with a lot of oversight by FDA.

Suspension is an action taken against the firm's license. In order to take a suspension, the agency has to show that there's a danger to health and that there's grounds for revocation, and I'll talk a little bit in another slide about what the grounds for revocation are. The danger to health determination is made by a committee of product specialists and medical officers who review the inspectional findings.

We don't take suspension lightly. We know, number one, it's going to prevent a company from distributing its product, and perhaps from making a living. On the other hand, we're also faced with there's a danger to health for someone who might use the product. The grounds for revocation in this instance are generally GMP deficiencies.

The suspensions legally stops the interstate distribution of a product. However, in reality, it could also stop any intrastate, within the state, distribution of a product because the conditions leading to the suspension are also conditions for which we could take an action under the Food, Drug, and Cosmetic Act to stop the intrastate distribution, and we're likely, if a firm doesn't agree once we've suspended a license to stop distribution on an interstate level, that we will proceed towards taking steps to stop the distribution on an intrastate level, because the same grounds exist.

What happens to a firm if we decide that we need to suspend the license? We can reinstate the license. It's not a rapid process, but we do give it a high priority within the agency.

What happens after the license is suspended? We're in a lot of discussions with the firm. They're to submit a corrective action plan. We review it. Again, we're in discussions. There could be letters back and forth talking about some deficiencies with the corrective action plan.

The firm will go into limited operations. They can manufacture their product at risk under this correction action plan, but they can't distribute the product. We'll come out and do a reinspection, and if the corrective

actions are sufficient to correct the problems, we'll review all the findings, and then we will reinstate the license.

Something else that could happen after a suspension is we could go directly to revocation. If a firm for some reason can't submit a corrective action plan, our option may be that we have to revoke the license. There are other agency actions we could take, such as an injunction or a prosecution.

I'd mentioned earlier that in order to do a suspension, you have to have grounds for revocation. Well, these are the grounds for revocation. They're in the regulations. I've put the CFR cite. Like I said earlier, most of the grounds lately have been for GMP deficiencies.

There are basically two ways that the agency will proceed towards a revocation, and it really depends on the circumstances at the particular firm at the moment. We can do a direct revocation. This can happen immediately after a suspension if we've identified that the reason the conditions exist for revocation are due to willfulness in the firm -- in other words, they're doing fraudulent activities like keeping double books and hiding one book from FDA and presenting us the book with the good data -- and if there's careless disregard for the regulations -- in other words, we've warned a firm multiple times that

they're not complying with the GMPs and somehow, for whatever reason, they can't get themselves into compliance, and it shows that they really don't have the attitude to come into compliance.

There's basically no opportunity to correct. Basically, we've said we're going to revocation.

It's a fairly long process. It's an administrative procedure. It doesn't happen overnight. The firm is given the chance for a notice of opportunity for a hearing and it's published in the Federal Register. They can request a hearing. If they request a hearing, then FDA has to review the request, the revocation gets published in the Federal Register as a final action, and that's when product distribution is stopped.

Now, if the conditions don't exist for a direct revocation, we can start on the process of a revocation by what's called a notice of intent to revoke. Generally, a firm has been warned that there are GMP deficiencies, for example. We don't have a danger to health. It's just they've had continual warnings. Basically, the notice of intent to revoke is the last chance for a firm to correct its problems. This action does not prohibit product distribution.

A chart on enforcement actions. I should have said that you're not going to hear much science out of me

at all, to the point that I probably picked the wrong format for a chart to show you, but it was the only one I could get to work in my PowerPoint.

But anyway, don't get the impression that the enforcement actions are dropping off, because if you add up the enforcement actions for the fiscal years, '97 was eight actions, '98 was nine, and '99 is one, but we're early in '99. I think a good point to take away is that while the warning letters have dropped, the other actions with greater significance seem to have either stayed the same or we have other actions coming into play here. I think that probably '99 and the year 2000 will be pivotal times to let us know if firms can come into compliance or if we have to proceed to other actions.

You might be asking, well, what conditions would exist in order for us to send a warning letter or to take an action? What I did was I evaluated 11 warning letters that we've issued recently. Of those 11, I counted how many had validation problems, how many had SOP problems, and whatever.

Also, I thought I'd pull a few of those out of the warning letters, so you could see that they're not technicalities. We don't want anybody to get the impression that if a firm fails to dot its I's and cross its T's, we're going to send them a warning letter. The

issues in the warning letter are generally significant. Some are very significant, as a matter of fact. So I thought I'd just run through a little bit and give you some ideas of what we found.

Six of the firms, or six of the 11, had validation problems and eight of the 11 had laboratory control problems. Generally, in here the deviations include things that relate to sterility. For example, media fills were not performed or, when they were performed, no one thought to do growth promotion testing on the media used, so they didn't know, if their media was growing, what they might be finding in their media fills. Media fills are conducted to make sure that a manufacturer can prepare a product under their aseptic processing procedures.

Air sampling to monitor the microbes during production was inadequate. For example, some firms in their aseptic area would manufacturer products, for example, in a particular part of the room, but the probes would be over on the other side, so they really weren't picking up the microbes.

Six of the 11 firms had facility issues. One of the requirements is that a facility maintain separate or defined areas or control systems to prevent contamination or mix-ups. We found things like materials for research in

products for distribution or stored with products pending final release testing. That could lead to someone in the firm grabbing the wrong product, thinking it went through final release when in fact it might just be a research product.

There was no assurance that the appropriate air pressure was maintained between mold production and general production.

In one firm, there were holes in the wall between the gowning room and the aseptic area for production, gaps around ceiling tiles in the clean room, and the light fixtures were not sealed, so there was the possibility that clean air and dirty air, so to speak, was flowing back and forth between the spaces.

Eleven of the 11 warning letters had SOP problems. SOPs either don't exist, they're not followed, or they aren't adequate. One example was there was no procedure for validation of the air handling system, including whatever test methods they were going to use to validate the system; acceptance limits -- for example, how would they know when their validation was unacceptable; and the frequency that they were going to perform the validation.

Five of the firms had record keeping problems. Record keeping problems, you have a spectrum. It could be

to the point where somebody forgot to put in a date to the issue where, again, we have separate record keeping books or systems and they aren't consistent. The numbers don't match when FDA starts looking at them.

For example, one of them, the moisture test results were recorded at 6.8 percent in a log book, and it's 5.6 percent on the raw material records. We get a little suspicious when records don't quite match up. Sterility tests for days 3 and 7 of incubation are not recorded, so we would wonder was a sterility test actually done?

Five of the 11 had investigation problems. For example, in one particular firm, 11 of the 21 finished product and bulk sterility test failure reports did not indicate any corrective action. When a firm has a sterility failure, we expect an investigation to occur.

There was also no follow-up to determine the effectiveness of the corrective action resulting from a sterility test failure. So it needs to go the whole gamut. You have a test failure, you investigate it, you implement a corrective action, and you go back and make sure the corrective action was adequate to take care of the problem.

Production and process controls, three of the firms. For example, one issue was the effectiveness of the sterile filtration was not established.

Just to continue a little further, nine of the 11 had equipment problems, failing to calibrate, inspect, or check equipment. This seems to be a big area, especially with cleaning procedures, because people tend to not do a very good job on determining the effectiveness of the cleaning procedure for removing any residual product or cleaning agents.

In one instance, we had shredded tissue paper and green material noted inside the laminar flow hood for the aseptic connections that they were making. I didn't ask what the green material was. I didn't look that much into the report.

Also, six of the 11 firms had component and container closure problems. The regulations require for this one to store components, drug containers, and closures in a manner to prevent contamination. One item that I saw was that the source material, which was a fish, was stored partially uncovered. In other words, the tail was out of the bag. So you kind of wonder what's going to happen to this fish until they get around to using it in their production. Lawn equipment and petroleum products were stored adjacent to shipping materials. So we tend to look at those things a little seriously.

Seven of the firms had deficiencies related to the regulations in the 600s. Those are specific

requirements for biological products, and those include things like not reporting errors and accidents to CBER, not reporting adverse experience reports, and not reporting changes to manufacturing to FDA and doing a license supplement.

I'm not going to go through the next, but they do exist.

Now, those were warning letter issues. What are the kinds of problems that would lead us to suspensions? Numerous GMPs, and again, the GMPs are egregious enough to make us think that we have a danger to health. I read some earlier. The examples I gave weren't all from one firm, but if that were all one firm, then we would probably start thinking about suspension of that particular firm.

Generally, when we look at things that put us over the threshold and into the suspension arena, they are things that would affect the sterility of the product, failure to assure aseptic processes, failure to investigate sterility failures, distribution of product prior to sterility testing, and inadequate facilities to the point where you can't manufacture a product that's going to maintain its sterility.

I do have some examples. For example, 11 lots failed sterility testing and there was no documentation

that retesting was performed.

A dual record keeping system for recording sterility test results contained numerous inaccuracies and inconsistencies. For example, an informal notebook stated that a retest was conducted after a sterility failure. However, the official sterility log showed that the lot passed, but there was no documentation that we could find that a retest was actually conducted.

Distribution of contaminated product, and to compound the issue, the distribution records were such that the firm couldn't even trace who had gotten contaminated product, so that they could do a quick and effective recall.

Failure to investigate after numerous environmental monitoring limits were exceeded. There were 12 sterility failures with Enterobacter and no investigation to determine the source or if any other batches of product was affected.

One item I didn't put on this slide was mislabeling and while labeling doesn't generally kick people into thinking about suspension, this was an element in one of the suspensions, because there were two vials of allergenic extract that were mislabeled. The 1 in 40 dilution was labeled as 1 in 400, and it resulted in a patient having an anaphylactic reaction. So while

mislabeling generally doesn't kick us into a suspension, mislabeling such as that is not something we like to see.

What are some issues we consider when we're going to take an enforcement action? It's a very difficult job when we're trying to make sure we protect the public health from unsafe and ineffective products, and also to make sure that the products that are needed to maintain the public health are available when they're needed. It's not a difficult decision when we have really egregious problems where we have contaminated product being distributed, but there are times when the decision is difficult on when to take an enforcement action and what particular enforcement action we need to take.

For example, we look at supply issues. We do look at is this manufacturer the only manufacturer of a particular product, and how is the product needed to promote the public health.

The Center for Drugs has a procedure which we also follow to determine if a product is medically necessary and we need to make sure that there's continued distribution, and how we need to work with the firm to make sure the product is safe and we can continue to distribute it.

I listed Alice Gerkhardt-Godziemski's name. She is the product shortage person in CBER. I also listed

her phone number. When folks are out there and can't get hold of a product, she's the one who will follow up and decide, well, why can't the product be obtained and is it an action that we've taken that has caused the product shortage and how we're going to deal with it.

Another issue to consider is that we've picked the appropriate action. We're not going to necessarily think about stopping distribution if that's not the most appropriate action to take. Many times, we want a firm to voluntarily correct their problems and we work with a firm to try and figure out how to do that.

We also look at the compliance history of a firm. Is this the first time they seem to be out of compliance? Obviously, we're not going to do an injunction the first time a firm has GMP problems. Usually, the first time they get a warning letter. It's considered a prior warning. So we look and make sure that the compliance history is such that we need to take the appropriate action.

Very quickly, I'll talk a little about Team Biologics. Team Biologics was formed in order to use the best of both worlds. Basically the ORA folks, the Office of Regulatory Affairs, or the field component of FDA, had experience in doing investigations with a GMP focus. The CBER inspections were probably more along the specific

product areas and what was in the license.

We thought how best to utilize both components, and so now you'll see joint inspections by the inspection cadre of ORA, and generally a CBER product expert will also join the inspection if they're available. If they aren't available to be physically on the site, they're at least available by phone, so that if the inspection team runs across something, they can call in and ask for guidance.

The goals of Team Biologics were to establish more timeliness, if there was a violative inspection, in trying to take corrective action, writing the reports and getting them written in a timely manner, and consistency across the board. We didn't want inspections conducted on the West Coast different from those conducted on the East Coast. And like I said earlier, there's a focus on GMPs.

Not only is there an inspection element to Team Biologics, but there's a compliance element as well. There are field compliance officers who coordinate very closely with CBER compliance officers.

Currently, all product areas except vaccines are the responsibility of Team Biologics as far as the inspections, and vaccines I think are due to be turned over to Team Biologics in October of '99.

Are there any questions?

DR. OWNBY: Any questions? Dan?

DR. EIN: Yes. I'm sorry I won't be here this afternoon to participate in the discussions, but could you perhaps tell us whether the rigorous application of the regulations that have existed but weren't applied until relatively recently is going to be applied to the producers of raw materials, the pollen gatherers, and so on? This is a matter of considerable concern to the practice community.

MS. CONN: Well, it is required that components which are raw materials that are going to go into a drug product are in GMP compliance, and I believe that we've sent a letter -- don't quote me on the date -- in '95 or '96 to the source material suppliers, saying that they were required to follow GMPs, and they are going to be inspected with a GMP focus in mind.

Now, obviously, you don't hold a broad material manufacturer to all the GMP requirements for a finished pharmaceutical product, but there are things -- SOPs, record keeping, making sure your product doesn't get contaminated with other things. So they are being required to follow GMPs.

DR. EIN: Thank you.

MR. BULL: I'm Thomas Bull. I'd like to speak to that issue.

It's my understanding that the Allergen Products Manufacturers Association has initiated a program

in which they send out their inspectors to inspect the source material suppliers, and that's in response to our '95 or '96 letter. We've discussed that with them on several occasions and I got information from them just recently that they have initiated a program along that line. So they will be responsible for insuring all the small source material suppliers are in compliance with the GMPs.

DR. WRAY: Who is that that will be responsible?

MR. BULL: APMA.

MS. CONN: Yes. That's another point I forgot to bring up, that the manufacturer producing the licensed product is also responsible for insuring that whoever they obtain their source material from also follows whatever GMPs are applicable and provides a product that meets their specifications.

DR. OWNBY: I had one other question. Have you thought about or had any estimates of what the impact of this is going to be on source material suppliers within the allergenic products? That is, the GMP application to source material suppliers?

MS. CONN: We've not sat down and done like an economic analysis or whatever, which was one reason why we felt it necessary to give adequate notice prior to coming

out and having to do a stronger enforcement action that would disrupt their supplying allergenic manufacturers, and also we didn't look to see how much money, for example, an individual source material supplier may have to expend in order to bring their firm up to what we would consider current good manufacturing practices. So we've not done an economic analysis, so to speak.

DR. OWNBY: Betty?

DR. WRAY: You mentioned there was a difference, though, in good manufacturing practices for these raw material gatherers versus the producers of the finished product. Do you have some examples?

MS. CONN: Well, the GMPs for finished pharmaceutical manufacturers go into things like how you have to maintain the sterility of the final product and all the testing that you do and all the validation. I perhaps should have used the word "difference," but to the extent of the GMPs, there are certain GMPs that a raw material supplier would have to follow. For example, like I mentioned, they have to maintain records, where they got the source material, how they processed it, they have to have procedures for processing it, but the procedures don't extend into the area of a lot of the sterility issues that you would expect from the finished product manufacturers.

DR. WRAY: Thank you.

MS. CONN: Dr. Hoffman?

DR. HOFFMAN: The point I want to make is that we've been sensitive to this issue, and that we have met with representatives of the Joint Council, we've discussed this with the APMA, and we've invited anyone to document any impact, either on a shortage with the compliance people or with us directly if they see that this is resulting in an adverse impact, either in terms of availability or, conceivably, in price, although that's not our major focus, that they bring this to our attention and we'll work with the compliance people and the manufacturers to see whether the rules in fact are onerous or appropriate.

I would say that we've invited people to demonstrate, tangibly or unpublished, evidence that these shortages are occurring or imminent to occur, and have so far received no response.

DR. OWNBY: Any other comments? Anyone in the audience want to comment on this? You do it at risk of everyone's hungry stomachs.

(No response.)

DR. OWNBY: Okay. Well, we are adjourned then until 1:15.

(Whereupon, at 12:17 p.m., the meeting was recessed for lunch, to reconvene at 1:15 p.m.)

AFTERNOON SESSION (1:20 p.m.)

DR. OWNBY: I think we're ready to get going again. We have one clarification of our discussion that we had just before we broke.

MR. BULL: I'm Tom Bull. I wanted to make a clarification about the inspection of source material suppliers and what the status of that is. I consulted with some of my colleagues in the industry, and it turns out that the Allergen Products Manufacturers Association, the APMA, did contract out to inspect two or three of the larger source material suppliers. At this point, each firm is responsible for inspecting their own source material suppliers, and we will be looking at that, these inspection reports and the fact that they're doing them, as we go on our inspections of the firms themselves.

So just like other parts of biologics, the firm has to be responsible that the materials it's bringing in for the manufacturer is appropriate quality, and the way they do that is by contracting out to have these source material suppliers inspected individually for each company.

DR. OWNBY: Okay. Thank you.

Next on our agenda was a report on clinical activities by Dr. Turkeltaub.

DR. TURKELTAUB: Hi. I'm Paul Turkeltaub. Can you hear me?

DR. FREAS: The sound man is going to be right back, and I don't know how to operate the sound board.

DR. OWNBY: He's coming.

DR. TURKELTAUB: Okay, is this better?

DR. OWNBY: Yes, that's it.

DR. TURKELTAUB: Okay. For the record, I'm Paul Turkeltaub, associate director of the Division of Allergenic Products and Parasitology. I wanted to give you some background on the clinical research program that has been developed over the years and where I'd like to see it go potentially in the future.

Let me just say that I first came to the FDA in 1977 when it was the -- should I flip it, or no?

DR. OWNBY: Let him switch it.

DR. TURKELTAUB: The one right next to it may have the same problem. Should I just wait? That's okay.

When I came to the FDA in the mid '70s, the aim was to develop a clinical research program that could validate some of the laboratory initiatives in standardization that Jay had mentioned, and the basis for the standardization program at FDA is not a discretionary one. The statute we work under, as Cathy Conn mentioned, one of the ones is the Public Health Service Act, and allergenic products are considered biological products, and as a Public Health Service officer, I certainly like

implementing the Public Health Service Act.

It says related to licenses that licenses may be issued only upon showing that the establishment and the products meet standards. So standardization is a statutory requirement. It also in addition states that the Public Health Service may prepare and distribute any product described in Section 351 -- i.e., that the Service may prepare and distribute U.S. Standards of Potency. This law goes back to 1902.

The panel on review of allergenic products which was mentioned in Steve Falter's talk, when they reviewed all the non-standardized products that were on the market, concluded "The panel therefore has recommended that the large proportion of these preparations be retained on the market and made available for continued use pending adequate standardization and further investigation to determine their effectiveness." So there's always been both the statutory requirement and the consensus of the advisors that these products need to be standardized, and that's been the aim of the program, the clinical laboratory program for the last couple of decades.

Now, what's the advantage of a U.S. Standard of Potency? It should be noted that when we approve a U.S. standardized product, like grasses last year, all the products labeled "No U.S. Standards of Potency from the

Same Source" are removed from the market. The value of the U.S. Standard of Potency is that it's a common unit, now in BAUs. That means it's interchangeable if the patients or physicians go from one health plan to another. It's very easy to switch from one company to another, depending on the availability. It's quite cost-effective because it's very easy to compute cost per BAU. This has a downward pressure on cost, which is a Healthy People 2000 and 2010 initiative for DHHS.

There's a tremendous value of information which, because it's applicable to all products labeled with the same unitage with respect to safety and efficacy, if patients read or physicians read that 100 to 500 BAUs is an optimal therapeutic dose, and 500 units and above is a maximum tolerated dose associated with increased risk, they can find out what dose they're getting and whether that's a safe dose or potentially a hazardous dose. Also, any complaints or adverse events related to a particular product BAU, when it goes into the database, all products can go into the same database. So there's a tremendous value because the entire product labeled with that unit information can be easily used by the physician and the patient.

Having a U.S. standard I think promotes innovation because it's easy to compare the innovative

product with respect to the standard product to see whether it's equal or superior. Also, the development of a U.S. standard relies on consensus with manufacturers and academia, so that there are defined criteria for what constitutes a standard. I should say that in Europe, where there is no Public Health Service Act, there's no FDA, that the European Pharmacopeia permits in-house or proprietary standards, which Daveck has characterized at the last Paul Ehrlich meeting in Bethesda at the NIH, that this has resulted in a proliferation of units, a jungle of units, great confusion among users, marketing claims that are more apparent than real, bondage that physicians are kept in to individual manufacturers because of the obvious risks in switching products which are not interchangeable.

I don't know if this is the marketplace you want in the U.S. I think this is what the statute requires. That's what I would support.

Now, with a proprietary standard, one has competition based on units per cost, leading to this jungle of units, increased number of units to differentiate products. There's decreased value of information because each manufacturer's product-related information is not applicable to any other products since they're all labeled with different units, which is very different when you have a common unit and safety and efficacy data tied to a

certain unit. Whereas a U.S. standard promotes innovation, I think that having proprietary standards promotes marketing.

And the manufacturer-dependent standard has what I think Jay Slater mentioned, which is what's the criteria for an acceptable standard if you have an in-house standard, and I don't know that the European Pharmacopeia puts any minimal criteria, whereas we have well-defined criteria.

Now, one of the chief aims of the clinical program when I came was how we were going to have bioassays in humans that define the potency, composition, and the biological basis for assignment of units to standardized products. The paradigm that fit very well in terms of termination of potency, which Jay mentioned earlier in his talk, is the concept of relative potency, which is simply the ratio of doses for identical response. The first approach to using the concept of relative potency was with the parallel-line assay, where the difference between these two parallel lines, these log dilutions, is the ratio of dose for the identical response. In this case, it's allergic response.

However, this is a graph from a paper by Gleich in the early '70s looking at RAST inhibition, where you can use parallel-line assays to look at the ratio of doses for

the inhibition response. So you can use the same paradigm of relative potency in vitro and potentially look at the relative potency in vivo. It should be noted that although this was done in 1974 by Gleich at Mayo, it was supported by FDA and Harold Baer, who was the chief of the Allergenic Products Branch at the time, who was one of the co-authors on this paper. In this paper they did correlate the difference between these different dose-response lines with the skin test reactivity and showed there was a relationship: the most potent required the smallest dose to elicit a certain size response.

However, FDA did not feel comfortable using the kind of skin test data they had at the time because it did not have defined accuracy or precision. So they clinically didn't feel they had a good basis for the clinical validation of the methodology. So in terms of developing the skin test relative potency assay, we used intradermal testing, which is a more sensitive approach and a more accurate delivery of allergen than percutaneous testing, and one can do a serial titration, which is standard practice, and outline the responses.

You can see that in these three-fold responses, that the wheal response is pretty flat even though this dose is nine times more potent than this dose, whereas the erythema response increases quite rapidly near the

endpoint, and we can make a permanent record for the notebook and then measure the sum and the longest orthogonal diameters to get an estimate of the intensity of the response.

As I mentioned, if you compare dose-response lines using wheal versus erythema, near the endpoint erythema dose-response is much steeper than wheal, and you compare the identical extracts to each other where the relative potency should be 1. A 95 percent confidence interval around parallel erythema dose-response is about three-fold, whereas they're about ten-fold using wheal dose-response lines. That's why the skin test assay we use relies on the erythema dose-response line.

These are the clinical criteria for an acceptable dose-response line. It has to have four serial dilutions, a graded erythema, a very high coefficient correlation exceeding 0.92, and the sum of erythema should bracket 50 millimeters, which is near the midpoint of the dose-response line, and include the endpoint, and this ensures a steep slope. So it has both clinical and statistical criteria for an acceptable dose-response.

One of the issues that we have to address is what was the quality of the data being submitted, and just like FDA requires clear for laboratory tests, we developed a proficiency program for clinical testing. Now, I don't

think any training program requires proficiency testing for clinical skin testing, but I think they ought to. We had a proficiency skin test method for the last decade, and I've asked investigators submitting data in support of standardization, or even just clinical trials comparing IND products in terms of patients, that they submit proficiency data in support of skin test data that they're submitting to ensure that they carried it out with acceptable accuracy and precision.

The approach we took was to take two known concentrations of histamine base, 1.8 with respect to 0.1. Here we know that the relative potency should be 18-fold. Then they can do within a subject a titration of each of those concentrations and get the dose-response lines, make certain it met the criteria for an acceptable dose-response line, determine whether the lines were parallel, and then calculate the horizontal distance between the two dose-response lines. In this particular case, let's say it's 18.2, and one can take the observed relative potency over the theoretical relative potency and get a handle on the accuracy of the test.

Based on data from many different clinical settings and operators, when you do four subjects with those histamine titrations, the observed value should be 65.7 to 152.2 percent of the expected or theoretical value

of acceptable accuracy, and your standard deviation mean should fall below this 99 percent upper limit to have acceptable precision. When somebody submits this data, then we consider them proficient in the parallel-line estimate of relative potency.

This is just an example comparing ELISA relative potency estimates based on the parallel-line ELISA inhibition curve versus skin test relative potency estimates. These are for the eight different grasses that were standardized as of last year. It doesn't matter which is which, just to say that the estimates based on the in vitro estimate of relative potency are very similar to the in vivo relative potency estimates.

We presented data like this from using RAST inhibition, showing that the relative potency by RAST inhibition is highly predictive of the relative potency by skin test in the mid '80s when we standardized mites.

Now, one of the major emphases in terms of allergy standardization -- King and Phil Norman were involved in isolating the first major allergen, Antigen E from short ragweed -- was to define the major allergen for FDA operationally. That is that the relative potency based on the major allergen content is particular to relative potency based on parallel-line skin test. Again, when Phil Norman published a paper showing that the content of

Antigen E was inversely associated with the dose required to give a 1+/2+ skin response, that paper was funded by FDA, and again Harold Baer was one of the co-authors.

Also, at that time, Antigen E at the time was not -- FDA did not move on requiring that for allergen standardization because --

DR. FREAS: Dr. Turkeltaub, I hate to interrupt you, but could you use this microphone instead?

DR. TURKELTAUB: Is that okay?

DR. FREAS: Is that any better in the back of the room?

DR. TURKELTAUB: Is it all right?

I was going to say that when Phil Norman showed that the Amb a 1 content was inversely associated with the dose of allergen required for a 1+/2+ skin test, again FDA felt uncomfortable proceeding with standardizing short ragweed based on Antigen E at that time because, again, the precision of the skin testing was not known. It was felt that the +/-1 10-fold dilution they were using, at least 10-fold dilution, so that was plus potentially 100-fold variability in the estimate, plus they weren't using a quantitative skin test. It was a 1+ skin reaction, which could vary in size from a 5 to 10 millimeter wheal to 10 to 20 millimeters erythema.

So we're talking about a very imprecise

clinical estimate at the time. However, when we developed the parallel-line skin test model based on the erythema response, then we could take a look at the relative potency based on Amb a 1 content with respect to relative potency based on parallel-line skin tests. This kind of data gave us an operational basis for labeling Amb a 1 a major allergen and using this kind of clinical data to support going ahead and standardizing short ragweed based on its Amb a 1 content.

Similarly, with Fel d 1, we had the opportunity to look at relative potency based on Fel d 1 content versus the relative potency based on skin test. It was a portion of the data we collected, and there was a nice association then too, showing that Fel d 1 was a major allergen.

As I mentioned in response to Dennis Ownby, Der p 1 is not a major allergen. We could find no correlation of RAST or skin test relative potency based on Der p 1. It was presented to the advisory committee, and they agreed on that basis to go with RAST inhibition as the potency assay. As I mentioned, in the Journal of Allergy in 1988, Der p 1 accounts for a negligible percent of the overall allergenic activity of D. pteronyssinus using either skin test or histamine release. It was about 5 percent, which was the variability of the assay.

According to Lowenstein in a recent chapter on

manufacturing and standardizing allergen vaccines in the Marcel Dekker book on allergen immunotherapy, major allergen content is not a measure of overall allergenic activity. You need to use RAST inhibition. In a paper cited by Jay Slater earlier, Haugaard, where they looked at the optimum dose of Der p 1 for immunotherapy, the methods section indicates that the total allergenic activity was measured by RAST inhibition, not Der p 1. Der p 1 is not a major allergen. It's not even a potency measure, if you talk about potency being the overall or total allergenic activity.

Then again, we have Dr. Lin's paper, which Jay Slater mentioned earlier. There's no correlation between the relative potency by using ELISA inhibition using a human IgE serum pool against D. pteronyssinus. There's no correlation with that and the content based on Der p 1. Der p 1 fell off quite rapidly in a very short period of time. ELISA inhibition in terms of its IgE binding stayed up quite well.

There's a whole bunch of allergens out there that are probably like Der p 1, and they could be whatever, you name it, and what I'd like to see is validation, that when somebody calls it a major allergen, that it in fact does account for the overall allergenic activity. That would be the paradigm I would use for defining a major

allergen.

With respect to compositional differences, we heard talks earlier, sophisticated approaches using MALDI-TOF or immunoblot approaches for looking at compositional differences in the lab. I was interested in looking at compositional differences, whether they're clinically meaningful or not. One way to do that is to look at the inter-patient variability of relative potency, and if it exceeds the upper limits seen with identical extracts, it's likely that that means those products are compositionally different. That means the relative potency is patient-dependent for compositionally different extracts.

But extracts which are compositionally identical, the relative potency should be identical regardless of the patient tested. We have an inter-patient variability of RP. It's 99 percent upper limit of SD. So if we see a relative potency estimate that exceeds that 99 percent upper limit, it's likely that that indicates lack of compositional identity.

We had an opportunity to apply this in a real-world application when looking at cat pelt versus cat hair extracts. Cat pelt contains cat serum proteins; cat hair doesn't. Cat serum protein does not contain Fel d 1, contains non-Fel d 1 allergens. So patients who are cat allergic but negative to cat serum are primarily Fel d 1

reactors. Cat allergic patients that are reactive to cat serum react to both Fel d 1 plus non-Fel d 1 allergens.

Then we looked at a pelt extract and a hair extract. The hair extract had 27 percent more Fel d 1 than the cat pelt did. The cat hair had 15 Fel d 1 units. As expected in the skin test estimates in cat serum-negative folks, these are Fel d 1 reactors, the skin test relative potency was very similar to the relative potency based on the Fel d 1 content, as would be expected. However, interestingly, in the cat allergic patients allergic to cat serum, the cat hair, despite having 27 percent more Fel d 1, had only one-sixth of the potency of the pelt. So that meant if the patient was on a cat hair extract and switched to a cat pelt extract that equalled Fel d 1 content, and that was a serum reactor, that person could be potentially overdosing, getting six times the overall allergenic activity.

In the opposite case, if the person was on a pelt extract and switched with a hair that equalled Fel d 1, the person would only get one-sixth of the activity that that patient had seen in the pelt, because the pelt contained so much non-Fel d 1 allergen. Based on this clinical kind of data, cat pelt was labeled not interchangeable with cat hair despite having similar Fel d 1 content.

Lastly, the clinical research program was used to develop a bioassay, a clinically-based method for assigning unitage to standardized allergens. Initially, the WHO -- and it still does -- uses arbitrarily 100,000 international units. Before we developed the bioassay method, we assigned arbitrary allergy units, 100,000, and then we developed an approach to biologically -- i.e., clinically-based units.

The unitage for biologically-based units should reflect the allergenic activity. Ideally, it should be common to all the allergens, and similar units should produce similar clinical effects in patients of similar sensitivity if it really works optimally.

The approach to defining potency using this approach was based on the dose-response line, which I've mentioned how we've defined, both clinically and statistically, and it's the dilution for a 50 millimeter response, where the D50 defines the potency of that product in that particular subject. And we also have a histamine proficiency program for knowing whether the person can target an appropriate D50 using histamine base 0.1 and 1.8, with 95 percent limits and an upper limit on the standard deviation. If they fall within these limits, then we can say that they're proficient with respect to D50 estimates.

The kind of subjects that we were interested in

evaluating were subjects who were likely to have severe disease related to the allergen of interest, and these were subjects with large skin test reactions by puncture test. We had access to the Second National Health and Nutrition Survey, which skin-tested a cross-sectional sample of the U.S. population in subjects with this size reaction. Sum of erythema greater than 75 millimeters accounted for less than 5 percent of all allergic subjects. So we took the 5 percent most reactive individuals we could find with the appropriate allergic disease, and we considered these to be the highest-risk subjects for both disease and adverse events if inadvertently overdosed.

We used the old Wyeth smallpox vaccine needle for the percutaneous test device. Now it's marketed by Allergy Labs of Ohio. We looked at a sample of extracts: short ragweed, perennial rye grass, white oak, English plantain, white pine, mountain cedar, and cat. We expected short ragweed with a high Antigen E and perennial rye grass to be very potent materials, and we looked at the mean D50, and they were sort of similar. We looked at white oak and plantain, and they were a couple of log dilutions, threefold dilutions less potent. As expected, white pine and mountain cedar in Bethesda are not potent allergens, and they were many logs less potent than these products. So clinically this kind of data worked with what our

expectations would be from a clinical experience, and cat seemed to be similar to white oak and English plantain, a little less reactive than perennial rye grass and short ragweed.

Although there are these marked individual differences, when you grouped weeds and grass and tree pollens, and cat and mite and cockroach alternaria, the mean D50s weren't all that different, which suggested that we wouldn't have a great chaotic distribution of potencies, that most, if manufactured appropriately, could be manufactured to have overall similar potency, which would be the aim of standardization.

We did a frequency distribution on D50s on 43 products, and these were the means at each of the dilutions, D50 dilutions, threefold dilutions. We found that the mode was at a D50 dilution of 14, which is 3^{-14} dilution for a 50 millimeter sum of erythema response. When you do 15 subjects, the 95 percent confidence interval is about +/-1 threefold dilution, and that's why these bars are at about 10-fold intervals, and why these are in 10-fold decrements. We labeled this mode as a reference group of allergens since it contained the most potent extracts, short ragweed with large amounts of Antigen E, and 1-20 weight by volume grass extracts. We labeled them 100,000 bioequivalent allergy units, and then based on the D50, the

decrements.

When we standardized mite extracts, that fell within the 10,000 range. When we did cat, it fell within the 10,000 range. When we did grass, initially it was above the 100,000 range, and we got concerned whether that was going to be too potent if we standardized grass at such a high range since we had a lot of experience at the 10,000 range. So when we looked at grass and presented it to our advisory committee previously, we decided that we would target 10,000 grass so it's consistent with mite and cat, but make 100,000 available if physicians wanted a higher potency for preparing mixtures.

Now, the concern I would have with cockroach and latex is that I'm not certain if latex is 100,000, a million, 10 million, or 100 million. I'd like to know what the D50 estimate is on that product before I feel comfortable with its potential safety in the U.S. population. Cockroach, again, our experience in the '80s was that it can be around 10,000, and some of our experience in the last couple of years is that maybe it's more like 1,000. Whether that's the best product, we need to work on that. Since Jay only came in the last six months, I think that question may be addressed in the future if these kinds of studies are ongoing.

When you look at 10,000 BAU extracts, and we

looked at the percutaneous reactivity in that very highly sensitive population, we looked at overall percutaneous erythema and wheal to the grass extract, standardized grasses to Df and Dp, mite, cat hair, cat pelt, and as we expected, the percutaneous reaction sizes are similar, which goes along with what we said if biological standardization works. An intradermal dose for a 50 millimeter sum of erythema response for all these different products is 0.02 BAUs per mL. If you take 3^{14} times 100,000 BAUs, it comes out to 0.02 BAUs as you go in 10-fold decrements.

This is important, 0.02, because if the doctor does a puncture test and looks at the reactivity of the patient tested, and looks in the package insert and it's similar to that puncture test reaction, then there's an intradermal guide in there about what kind of intradermal responses were seen in very highly sensitive patients, and this is the mean kind of response. You also look at the literature, and patients who react at this level of 0.02, which is about a 1-to-5 million or thereabouts dilution of the concentrate, they are at high risk of having serious adverse events if overdosed, of having more disease more likely to be related to the allergen you're interested in immunizing with, and also these patients are more likely to respond beneficially to immunotherapy. So it has important

prognostic/diagnostic value.

Maybe we could flip this slide in the right direction.

DR. WRAY: Excuse me. While you're doing that, could I ask whether -- you'd mentioned that mountain cedar was so much less reactive in people here. So where were these people from? Did you take that into account?

DR. TURKELTAUB: We have Juniper Virginianis, Virginia cedar, locally. So I assume that's cross-reactivity because it's a local -- we don't have mountain cedar here.

DR. WRAY: Right.

DR. TURKELTAUB: So that's why I expect it to have less reactivity, just like white pine.

DR. WRAY: But when you're standardizing, do you use people just from this area, or are you looking --

DR. TURKELTAUB: No. If we were going to standardize mountain cedar, which I think would be interesting to standardize, we would go to where mountain cedar is.

DR. WRAY: Thank you.

DR. TURKELTAUB: In terms of the clinical research program proposals, what I'd like to see in terms of determination of D50 for the candidate latex and cockroach reference extracts, we're trying to develop that

data. Since Jay has only recently come, we're going to see if we can get some clinical sites to actually generate that data now that we have a lab that can evaluate our working reference and make certain it's satisfactory, which it seems to be. The question is whether this approach will be applicable to future references.

I'd like to validate whether some of these allergens that claim to be major allergens are really major allergens in that they are predictive of the overall allergenic activity.

I'd like to see whether some of the assays that we're looking at in the lab, to see if compositional identity is, in fact, clinically relevant.

I'd like to see studies of diagnostic and therapeutic safety and efficacy carried out, like we did with some of the studies that Jay mentioned earlier that I was involved with in terms of short ragweed. I think the most important element here was doing diagnostic studies of safety and efficacy, because there's been a tendency to do therapeutic studies without first knowing whether you can actually diagnose the person accurately.

In addition, we're carrying out studies with the Centers for Disease Control using the Third National Health and Nutrition Survey to look at prevalence of allergic disease in the U.S. We skin-tested I think 30,000

U.S. citizens as a sample of the U.S. population to look at percutaneous hypersensitivity to standardized allergens. We're interested in analyzing that data. That's just starting now.

We're doing a mortality follow-up of the allergy portion of the Second National Health Survey to see if atopy influences mortality. We've finished up a CRADA with the Center for Devices and CDC on a seroprevalence study of IgE reactivity using the Third National Health and Nutrition Survey. We're doing a study with the National Center for Health Statistics on the impact of allergic disease on fertility since we know most of the allergens people are hypersensitive to are reproductive proteins, and we know TP is reported on homologies in some of the insect venom allergens with reproductive proteins.

What's not on here, and probably one of the most important issues with respect to adjuvancy, is whether the worldwide increase in allergic disease is related to exposure to a Th2 adjuvant. The developed world is exposed to aluminum, and whether that is having any impact on the increasing prevalence of allergic disease worldwide.

DR. SAXON: Can I ask you before you go on, I wasn't clear if the NHANES III is still going on.

DR. TURKELTAUB: No, it ended in 1994.

DR. SAXON: So under 4A, I wasn't quite clear

what you meant.

DR. TURKELTAUB: We're only now getting the data to analyze.

DR. SAXON: So it's all been tested, you're just crunching.

DR. TURKELTAUB: Testing was 1988 to 1994.

DR. SAXON: Okay, that's fine. And what was the IgE seroprevalence? I wasn't clear. Do you mean just the serum levels of total IgE?

DR. TURKELTAUB: No, total and specific.

DR. SAXON: I see. Okay. I got it.

DR. TURKELTAUB: To many of the materials that were skin tested, to look at the relationship between the IgE and the skin test reactivity, and the history.

DR. SAXON: Thank you.

DR. TURKELTAUB: Any questions?

DR. OWNBY: Any additional questions for Dr. Turkeltaub?

DR. UMETSU: This is a question I asked Jay already, and I'm getting a little confused. In terms of the standardization of, say, grass and perhaps cat, what measurements are you using to standardize it? Is it the skin test reactivity as you had talked about, or is it ELISA data that Jay had talked about?

DR. TURKELTAUB: Are you talking about what the

manufacturers do with each lot of material they manufacture, how they know what the relative potency is compared to the prior lot? Or are you talking about how we define what our U.S. standard originally is?

DR. UMETSU: Or both. Both.

DR. TURKELTAUB: Well, it's different, because the manufacturers are not required to do any clinical testing. We've done the clinical testing and validated our in vitro assays so that we know that they're clinically relevant, and then we give it to the manufacturer off the shelf and say, "This is a validated in vitro assay, you can use it for subsequent lots since we have already validated that it, in fact, predicts the clinical relative potency." So the manufacturer uses the in vitro relative potency estimate which has been validated as being clinically relevant.

DR. UMETSU: So you've compared directly the skin test reactivity and ELISA assay, and they come out to be --

DR. TURKELTAUB: Well, I showed one slide, and we had limited experience at the time that we did it, where we had ELISA relative potency in that bar graph, and in vivo relative potency, and they were quite consistent.

DR. UMETSU: And you've done that for all the antigens?

DR. TURKELTAUB: Well, the only antigens that we have are Fel d 1 and Amb a 1. We did it for Der p 1 and rejected it because it didn't correlate. The rest have been RAST inhibition for mite, and now ELISA inhibition for grass. For latex or cockroach, presumably it will be ELISA inhibition because that's probably the best overall estimate of allergenic activity, and I'd like to see some clinical validation of those estimates, that they are, in fact, applicable. I would suggest they would be, but I'd like to see the data.

DR. OWNBY: Any other questions?

DR. TURKELTAUB: T.P.

DR. KING: It's actually about grass pollen allergen. Aren't the number of major allergens really limited in the grass, or they are now reliable also?

DR. TURKELTAUB: You know, T.P., I'm a skeptic. I'm only convinced by data, and I haven't been able to generate -- for one, I don't have access to all the reagents.

DR. KING: That may be a problem.

DR. TURKELTAUB: Two, I'd need a nurse to do the studies because you need a clinical person who is dedicated to quality control kind of stuff, and at this point we don't have such a person to do the kind of clinical studies that could be done in the future, if that

was the way we were going to go in the future.

DR. WRAY: My question was about grass also. I thought that the titrated erythema was the basis on which the grass pollens were standardized.

DR. TURKELTAUB: In terms of our U.S. standard?

DR. WRAY: Yes.

DR. TURKELTAUB: In picking the standard, when we looked at assigning units, what got 100,000, what got 10,000, that was based on those D50 estimates. The D50 is the dilution for 50 millimeter sum of erythema response, a 2+ skin reaction.

DR. WRAY: And were all those subjects from this area?

DR. TURKELTAUB: No, not all, because Bermuda came up as being less potent in our hands, and so the advisory committee asked us to replicate that data in an area where Bermuda was a much more important allergen than in our area. We went to Vanderbilt University and John Murray replicated the D50 estimate for Bermuda where we found it.

DR. WRAY: So that's how we wound up with two different ones, one labeled 10,000 BAU and the other one labeled 100,000. Clinically, we see them identically. We haven't done the titrated erythema, but just in terms of pricks.

DR. TURKELTAUB: Well, I think puncture testing, depending if you're looking at wheals, everything looks pretty flat. You're not going to be able to make distinctions with wheals. You're going to need to have well-defined titrations to pick up the differences we're talking about. Unless you do it this way, you're not likely to detect a difference the way you're doing it.

DR. OWNBY: Any other questions?

DR. TURKELTAUB: And I think with Bermuda it's been shown in in vitro studies that it was less active. I think Bernstein did a study way back when people were doing a lot of RAST inhibition studies with different grass extracts that Bermuda was less active in RAST inhibition than the other grasses were.

DR. WRAY: Okay.

DR. OWNBY: Thank you, Paul.

We've managed to drift a few minutes late, but I think it's now time to open the public hearing.

Bill, would you like to update us on who has requested time to speak?

DR. FREAS: Sure. As part of the advisory committee procedure, we hold an open public hearing for members of the audience who are not on the agenda to have a chance to participate in presenting issues before the committee relating to today's topics. To date, I have

received one request to speak before the committee, and that is from Dr. William Storms from the Joint Council of Allergy, Asthma, and Immunology.

Dr. Storms, would you come to the podium at this time?

While he's approaching the podium, I asked for him and all other speakers to please address, in the interest of fairness, any current or previous financial involvement with any firm whose products you may wish to comment upon. Thank you.

DR. STORMS: Thank you. I'd like to thank the committee for the opportunity to give this presentation. The discussion that I'm about to embark upon relates to the package inserts for inhalant allergenic extracts. The Joint Council of Allergy was prompted to propose that a committee be put together, of which I am the chairman, to discuss these package inserts because different manufacturers, as you know, have different package inserts, and these differences may make it confusing not only for physicians but also for patients. We felt that it may even create a safety risk because of the different constructions.

The committee was myself, Dr. Don Aaronson, Drs. Robert Miles, Tom Fisher, Hal Nelson, and Peter Credikos. We have met by conference call various times

over the last two years to look at these package inserts and try to come up with something that we thought we could put forward to your committee as a proposed inhalant allergenic extract package insert. You have that document in front of you.

What we did was we took many of the items from the current package inserts and then tried to put them in a format that would be somewhat flexible and could be adapted by different extract manufacturers for different allergenic extracts. I'm not going to go through the document. I think it speaks for itself. I'd be happy to answer any questions.

DR. OWNBY: Are there questions for Dr. Storms?

(No response.)

DR. OWNBY: What would you like us to do with this document?

DR. STORMS: Well, if you deem it to be something that would improve upon what we have out there now, then I think it would be -- you might consider it appropriate to send it to the extract manufacturers as a draft or a format by which they could then -- this is for inhalant allergens -- by which they could modify it for their own extracts.

DR. SAXON: I have two copies here, one from November 24, 1998, in the packet, and another from the year

-- actually, 19,999. I just wanted to make sure they were identical. Do you know?

DR. FREAS: I can explain the difference. The one that you received in the mailout -- Dr. Storms was planning on making a presentation. When he decided to make a presentation, he sent us a more recent copy, which I believe is the same, but I'll let him decide on that one.

DR. SAXON: I was just trying to make sure it was the same because I don't want to go over the different points if they're the same one.

DR. STORMS: Well, I'd have to go through and look at it, but it appears to be the same.

DR. SAXON: Okay. We should be working from the January one?

DR. STORMS: I'd go with the January one.

DR. OWNBY: Do you have any instances of practitioners reporting problems because of the differences between manufacturers in the current product inserts? I mean, I'm sure your committee didn't decide just to make work for itself, but I wonder how strong the impetus was to try to come up with a standardized insert.

DR. STORMS: Well, we haven't, to my knowledge, had any bad reports, so to speak. There have been some concerns about changing extract manufacturers in midstream. In another words, if a given clinic or managed care

organization buys from one manufacturer, and then on January 1st they get a better deal from the other manufacturer, they switch everybody to the other manufacturer, what are the potential implications for the patient?

In this document, you'll see something that isn't in most of the package inserts, and that is some guidelines for changing dosages if you switch from one manufacturer to another, or if you switch from an extract that has been expired a little bit, the same manufacturer with a fresh extract.

DR. OWNBY: Would any of the people in the audience like to comment on this? I'm sure there are several manufacturers who have read through this previously. Does anyone want to comment? Good idea? Bad idea? You don't want to know about it?

DR. STORMS: And the document was sent to whatever group it is, the Extract Manufacturers Committee -- is that what it's called? -- for review.

DR. MARABEL: I'm Dave Marabel from Bayer. I believe it was reviewed in part through APMA. From Bayer's standpoint, I think we're a little cautious on this because this isn't a generic industry. It's an individual industry of various manufacturers, and I think from Bayer's standpoint we want to make sure before we get into this

that our identity and uniqueness is preserved in this insert.

DR. STORMS: There are areas within this recommended package insert where each manufacturer can put information relating to their own extracts and what differentiates them from other extracts, for instance, and what the different concentrations are. Maybe that should be more flexible. We didn't receive any comments back from that group, by the way.

DR. SAXON: I have a question for maybe the FDA representatives here. Even drugs such as beta blockers, they're very similar but have very different package inserts. Would this kind of approach be something that has precedent, or would this be relatively unique for the FDA to come out and say, "We're going to set the block for this group of materials"? I'm just not familiar with whether this is precedent-setting or just old hat.

DR. HOFFMAN: It's certainly not consistent with my experience. It's not something that we encounter. I've not encountered it previously. I don't know if anyone has.

Martha or Paul?

DR. TURKELTAUB: I agree that we have never mandated boilerplate labeling. Everybody has been permitted to -- in the Code of Federal Regulations it has a

content and format for regulating, but the companies put in each of the subheadings what they feel is appropriate, with guidance.

DR. SAXON: Yes, and they have their own council to help them with their own vantage point.

DR. TURKELTAUB: So I think maybe the most useful thing is if there would be highlighted some particular elements, as opposed to a boilerplate that is missing. In reading this, it seemed like a lot of the items are already in a lot of the inserts. Now, if we can respond to specific instances where they aren't in inserts and ask the company to put it in there, as opposed to saying "Use this verbiage," but I'm not aware that it's missing. I don't know which companies it may have been a problem for.

DR. SAXON: So I guess what you're saying is that if they could identify what they thought the critical elements were, it would be better than trying to provide a boilerplate, which would be a little unusual for the FDA to say, "Write this," rather than saying, "You should have these six key elements covered," and then the manufacturers would go back.

DR. TURKELTAUB: Exactly. As far as I know, I'm not aware that any insert doesn't cover this.

DR. SAXON: Well, there is an issue, obviously.

The Joint Council felt it important to find out exactly what those key issues are, rather than trying to mandate a boilerplate for an industry.

DR. HOFFMAN: My surmise is that if the Joint Council worked with the manufacturers to help them provide language in their package inserts that would be more general that would be consistent with what they were trying to achieve, then it would behoove the manufacturers to then amend their licenses and put it in the package insert consistent with that language. It wouldn't be an impediment for our reviewing the same package insert from each company, but I think they would have to be reviewed as part of each license.

DR. SAXON: There's only nine manufacturers? Is that right? How many?

DR. HOFFMAN: Well, I think there are different allergens that not every manufacturer manufactures.

DR. SAXON: But I meant for inhalants in general. This is just for inhalants, right?

DR. STORMS: Inhalants, correct.

DR. SAXON: So there are only, I think -- I heard today on the order of nine. I don't know if that's correct.

DR. WILLOUGHBY: Hi. I'm Tom Willoughby with Antigen Laboratories. There are a couple of things that

this package insert doesn't address. It doesn't address the many types of skin testing, RAST testing-based treatment modalities. Also, with the companies that do extract directly in glycerin and don't do an aqueous extraction and put the glycerin in later, we don't have the variability that some of the other companies do in their products, which is not addressed in this.

DR. STORMS: To your first point, we didn't want to get into a full practice parameter on skin testing, or a guideline or something like that -- excuse me, on allergen testing, whether it be skin or in vitro. But we did reference the practice parameters on allergen diagnostic testing in the references for those people who wanted to look that up.

To the second point, the section -- I think it's on the first page under "Description." There's a point that's made, "Each company may insert a description of their extract in this space," and that would be meant to allow you to identify if it's glycerin or how you extract it or what the constituents are.

DR. OWNBY: Any other questions or comments for Dr. Storms?

DR. WRAY: I was just going to comment that I think the first line is one that's very important to allergist immunologists, but I'm not sure whether it's in

the current package inserts or not, and that is that "This product is intended for use only by physicians who are experienced in the administration of high-dose allergen injection therapy," because I think the pressure is on by many insurance companies and managed care for non-allergists to prescribe immunotherapy and supervise the administration of it. So that may be one of the key elements, and I'm sorry, I don't know whether it's already in the package insert or not.

DR. STORMS: In some of the newer, more recent package inserts, especially the standardized animal danders, there is a statement similar to that. It isn't quite the same. In other ones, it's not. I don't want to name any names, but I just picked out five from our clinic that we ordered from in the past few years, and there's no comparison between these five different companies' package inserts.

DR. OWNBY: Other comments or questions?

(No response.)

DR. OWNBY: This is now part of the public record. We can certainly consider it. Thank you very much for your presentation.

DR. STORMS: Thank you very much.

DR. OWNBY: Are there any other individuals who would like to address the committee during this open

hearing section?

(No response.)

DR. OWNBY: Okay. Then hearing no one who wishes to speak, we're going to take probably a 10-minute break at this point, and then we can -- do we have any other items under committee discussion at this point?

DR. FREAS: No subject items, no.

DR. HOFFMAN: We do want some feedback from the committee. Whether we do it before or after the break is not important. I have one slide that can focus the discussion somewhat, but we are interested in some immediate feedback, if there is any, on the presentations so far in open deliberation.

DR. OWNBY: Okay. Then why don't we take a 15-minute break and come back at 2:30, and then if you'd try to start the committee discussion, hopefully then we can move into the closed committee discussion and adjourn on time, if not a little early.

(Recess.)

DR. OWNBY: We're opening the discussion section. Dr. Hoffman wanted to address a few issues here for the committee to discuss.

DR. HOFFMAN: This basically serves as an outline for one way to approach the discussion and highlights some of the points that we touched on today. I

have not labeled these as questions or decisions to be made, but rather issues to discuss. We hope that we've conveyed to you that in the last year, at the very least since we spoke and maybe going back to the last time we met, the agency has done a lot to put some of the allergenics regulations in some sort of order. We've brought in some different personnel. We've made some managerial changes. I alluded to some that we entertained.

We think that things are working fairly well. In the background there are issues of resources that Dr. Feigal touched on, there are issues of personnel. We can't ignore those, and we'd be willing to discuss them as you think them relevant. But at the same time, one of my points in raising this discussion is that I think we've shown you that we intend to remain at the forefront of allergenics regulation and to continue our standardization efforts, and Jay elegantly outlined his plans for doing that in the laboratory.

We're working very hard with the other parts of the center and the agency in general to make sure that some of the centrality that we were able to accomplish when everything was just under our thumb, so to speak, that's changed as well. Team Biologics now do the inspections. We're participants, and we help, and we're advisors, but we're not always the ones that are doing the inspections,

nor do we have the resources to do every single inspection that is necessary. So we've tried to use a little bit of cooperation, collaboration, managing some of our resources, I guess the jargon is leveraging them, to try to do the best job we can.

Some things we can do, some things we can't do. We have some ambitious programs. At the very least, we certainly don't want to lose any of the gains that we've accomplished with the wonderful standardization effort that was brought to bear about one year ago. We'd entertain any comments, critiques, criticisms or suggestions for doing things differently. We can follow some of the sequence that I outlined, or we can be random based on what stimulates your interest. I'm going to sit down and listen.

DR. OWNBY: Well, I have one question for you before you sit down. Maybe everyone else is clear on this, and I'm sure you are, but what is the relationship between CBER and DAPP, if you will?

DR. HOFFMAN: Okay. Sorry. That's just jargon. CBER is the center. The center encompasses five or six offices that do everything from therapeutics to blood regulation. We find ourselves in the Office of Vaccines. There is an organizational chart, but the Office of Vaccines, headed by Dr. Egan, is responsible for all

vaccine issues, including issues pertaining to allergenics vaccination, if we want to use our current terminology. So we are in the Office of Vaccines.

The division, DAPP, was created in 1992 at the time of a center-wide reorganization, and that incorporated existing components and was given the responsibility of allergenics regulation at that time. DAPP is the Division of Allergenic Products and Parasitology, but as I showed you in my introduction, it's not limited to that. We do some HIV work, we do some hepatitis work, and many other things. Obviously, the cornerstone of our efforts and our major regulatory activities center around allergenics, certainly for licensed products.

DR. OWNBY: Do any of the other committee members have questions at this point for Dr. Hoffman?

DR. UMETSU: I just have a question somewhat in response to Part 2, the research program. It sounds like Jay Slater is establishing a very nice, I would say, I guess intramural kind of research program. Is there any discussion about having some kind of extramural research program that might help in promoting the standardizations in labs that have more expertise?

DR. HOFFMAN: Yes. In a sense, however, we are not a typical funding agency. In other words, we are not the NIH. However, we do sometimes conduct studies that we

contract for. So in that sense, we do give certain grants. There are grants within the FDA for many things, and they would not necessarily be limited to allergenics. Usually the research program of the division is an intramural research program and supports collaborations, and occasionally contracts are let. But that hasn't been the key aspect of our research activities.

However, I think the point is very well taken. It's possible that we could contract some more, and possibly enlist funds for that. But usually when funds are solicited, it's based on either allocations on per capita or certain proposals that we put forward to our funding authorities that are usually controlled by the FDA.

DR. SAXON: I'd rather be random than organized. I'm trying not to get invited back. Put that on the federal record.

(Laughter.)

DR. HOFFMAN: You know how hard it is to be random.

DR. SAXON: What I was going to say is that you asked for what we think. I think you're very fortunate with Jay Slater here now. I think what you have with Paul Turkeltaub is very important, though, because, as you pointed out, without a biological assay, all of the fancy assays are not going to be important. You've got to keep

them together.

I think one mistake at this juncture might be to get too far afield into basic science. That has come terribly to me, who is a basic scientist who works on gene regulation. But what you've got is relatively unique. You've got people who are really focused on very important issues that we use every day in our lives as clinicians and want to ignore in our laboratories, and no one is going to do this but you folks. So I think to get too far afield into basic immunology and antigen characterization by molecular techniques -- what's the point? You can lose the focus, unless you get a huge infusion of money, which obviously we all wish for.

So I think you ought to play to your strengths there. The other step with that would be the idea of the advances in analytic characterization, and T.P. can probably speak more to that than I can, but other methods. I mean, we're really using methods that are 20 years old, except for a few things Jay mentioned today. I can't remember the branch of the NIH that has all the fancy instrumentation. They work specifically on one molecule coming off from another molecule for binding of single molecules. But if there could be new ways that would be the next generation, rather than getting involved with things like antigen presentation -- I think you could get

lost in that, myself.

I think I would focus on your strengths and your charge. I think that's where I would go.

DR. KING: But to follow what you just said, Andy, isn't that the allergen characterization? Otherwise, it's too complex a mixture for them to decipher, to regulate. They've got to settle on two, three, four, five. They've got to pick some of them. Otherwise, all the mixture contains at least 20, 30, 50 proteins in there.

DR. SAXON: So what are you suggesting, though?

DR. KING: My problem is that they say they found a major allergen. Most of these people run an SDS gel electrophoresis and run an IgE binding, and they say they found a major allergen. That's a lot of hogwash, because they have already admitted that the protein has been denatured. So they have to think more in terms of -- I mean, it is important to have a certain amount of research, but I also agree with you. FDA performs a vital role to regulate the products. Otherwise, the doctors in their office don't have any way to control it.

DR. SAXON: Do you think they should go for a few key antigens within an allergen mix, or do you think they should go for new technologies? I really don't know.

DR. KING: What do you mean by "new technologies"? You've got to know what you want to

analyze, so you have to characterize the allergen. You can't characterize all of them because you only have so much resource. So you've got to settle it down and say we'll pick the ones that at least 50 percent of the people react to, and we arbitrarily designate these are the major allergens, and then we've got to select a panel of them, not one. Only in some unusual cases, like the cat and the ragweed, they seem to work.

DR. OWNBY: But don't both of you think that there's a lot of research work being done on identifying allergens? Part of what this laboratory has to do is to sort out all of that information that's being generated and try to validate, as you say, which ones are truly measurable and useful allergens in the context of standardization and which ones may be minor and of relatively little use.

DR. KING: Right. If they can validate and also get the cooperation of these scientists to give the gene, then they've got it.

DR. OWNBY: But I think the committee that's here would have a consensus that certainly we want CBER to still be in the business of establishing national standards. I don't think there's any question about that, and along with that is maintenance and distribution. The ability to have standards that are meaningful means that

there has to be, as Andy said, a biologic basis for all of this.

Betty?

DR. WRAY: I would just add at the same time that any progress that can be made on some of the urgent clinical problems -- and I know it's hard to sort that out. But like latex, for example, right now, I personally don't know why there's been a delay in penicillin. There may be good reasons for it, but a few of these food allergens right now are causing deaths in children, even. But food immunotherapy hasn't come along. So I guess these are some of the urgent clinical things that we see every day that anything this lab can do to help move along some of that progress would be perceived as very helpful.

DR. HOFFMAN: Jay, do you wish to comment?

DR. SLATER: Yes. I think that I certainly take seriously everything that each of you said. I think it certainly is my plan to collaborate outside of the lab as much as possible, and I think that certainly I recognize that even if all of my dreams came true in terms of support for the lab, we simply wouldn't have the resources and the expertise to do everything that we'd like to be able to do in order to do it well. So certainly I have every intention of collaborating. That's not the same thing as saying you have an extramural program, but that's certainly

the direction in which I'd like to go, and we're planning on doing that.

I think that your thoughts about pursuing the technologies and the best ways to approach the regulatory responsibilities is a really major part of our activity. There's no doubt that that's going to be a major part of what we're going to do. I think one of the dangers of the kind of presentation I gave this morning is that everything sort of sounded like it had equal weight in terms of what direction we're going to go in. My guess is that's probably not going to happen, and my guess is that the research program is going to be, in a sense, driven by our regulatory responsibilities. It's probably going to hold great weight, and those research questions that arise from our regulatory jobs are really going to be the ones that get pushed forward the fastest and the hardest.

I couldn't agree with you more about not trying to characterize every allergen. I think that probably the most sensible approach, though, especially in terms of the glycosylation question, is going to be to identify good paradigmatic allergens, ones that really, by example and detailed study, can give us information about a whole class of allergens behind them, and I think that was sort of my purpose in discussing the avocado allergen, even though I really have no particular interest in avocado. But it

seems to be different from hyaluronidase in certain peculiar ways, and I think those differences can be explored as examples. But clearly, you don't want to track down every allergen that somebody says might be a major one.

I couldn't agree more about the urgency of certain clinical problems. I think it was my thinking that cockroach was probably the next likely candidate after latex on that basis, on the basis of the clinical urgency. Certainly we're open to the possibility that we might want to pursue some food allergens as well, but I think there's really pretty good evidence that the cockroach is something that probably will support a good asthma effort, and we probably need to have better characterization of that.

I was actually wondering if anyone on the committee wanted to reflect on the protein and the release limits discussion from this morning.

(No response.)

DR. OWNBY: It looks like a real exciting group.

(Laughter.)

DR. OWNBY: I think that the protein assay question seems to have been beaten to death several times. It almost seems to be unsolvable, but the approach you outlined, at least in my mind, seems practical, and for the

purposes that you're using, probably as good as can be done. I think your release limits make sense, and one of the things that I'd written down that I hoped the committee might want to comment on is that while you are trying to establish some potency limits and you talked about the therapeutic and the diagnostic and the safety, and I think all of us recognize that first and foremost should be the safety issue and how difficult it is to get even marginally adequate data, because you have to give a lot of allergy shots in a number of different ways to try to come up with the reaction rate and how it changes with the potency of extracts, because there are so many other variables that are part of that.

Do any of the other committee members -- I don't know, I think that's something that ought to be done, although on a practical basis it's extremely difficult. What you really need is a few huge HMOs that are willing to participate where you know that they're using standardized extracts in a similar fashion that can feed you data.

DR. SAXON: I don't see how you're going to get the data even then. I mean, what you really want to do is just take people and give them different doses, overdoses in a sense, and see at what point you start getting a hit. You just can't do it ethically. You've come up with that fourfold number. I mean, it's an interesting number. You

generated it, and it was clear how you generated it. I don't know how to validate it. But at least it was a smaller number than the numbers for therapy and -- what was the second one?

DR. OWNBY: Diagnostic.

DR. SAXON: Diagnostic. It was a smaller number at least. So at least safety has the smallest margin of error. I mean, you explained very well how it was generated. We realize it has feet of clay, but it was better feet than I could have thought of, I assure you. If someone said you're going to have a fourfold change in potency in this material when you inject this patient, would I panic? Probably not. It's the maximum. At tenfold, I'm getting worried. A logfold, I'm starting to get worried. But it's purely anecdotal, and the plural of that ain't data.

So I thought you did the best with what you've got, and I don't see how to get a lot better. As you say, how are you going to take 50 people and say, "We're going to give you this much, and next week we're going to give you fourfold as much, though it's not what you need"? You can get the data, but not through our IRB.

(Laughter.)

DR. OWNBY: Other comments from the committee?

(No response.)

DR. OWNBY: Jay, did you have other areas that you specifically wanted feedback?

DR. SLATER: No.

DR. OWNBY: I had written down this issue of the stability of extracts that Jay presented first this morning. It almost becomes a circular argument because if all the assays we're using are based on a reference standard, and when you try to understand the stability of your reference standard, is lyophilization the best standard that we know of? That you take a large batch and lyophilize it and say that that is now stable for a relatively long period of time that you can compare all these others to?

T.P., what do you think?

DR. KING: That depends. Actually, if you have just a pollen mixture and you lyophilize it, it's fine. If you have some purified protein you lyophilize, with some of them, that's the end of it. But some proteins can be lyophilized and stored for a long time. In general, the crude ones can be stored.

DR. SAXON: How about -70, T.P.? We have a real problem with lyophilization.

DR. KING: It's not -70. It's a matter of the process of lyophilization.

DR. SAXON: I just meant freezing at -70. Is

that better? I've had real problems with lyophilization.

DR. KING: Freezing is okay.

DR. SAXON: Because with some proteins, they're gone.

DR. KING: All the allergy extracts you use contain phenol in there, and the minute you freeze it, that's it, because the phenol gets concentrated down.

DR. SAXON: Right, but I was thinking for standardization. One of the problems is every time I take out of my freezer whatever the allergen is, if some have been lyophilized, they're gone. But we can freeze them at -70 in plain buffer.

DR. KING: Fifty percent glycerin is really a very good preservative.

DR. SAXON: And freeze it at -70?

DR. KING: That's really good.

DR. SAXON: Is that something that CBER should, that DAPP should look at, what's the best way to store the damn things? I don't know. You brought it up with your lyophilization at -70, Jay.

DR. SLATER: You know, I think it's a very fair question, and I think it's one of the things that -- and I'm not going to take credit for this study because this was really designed and initiated before I came on board, but I think that was one of the strategies in comparing

lyophilization to -70 to -20 to 4 degrees, to see what was actually happening. Your point is well taken, though. In the relative potency assay, we used the lyophilized product as our standard for the relative potency of 1, and in the specific allergen assays, the standards are just kept at 4 degrees. So there is a bit of circular logic there that's hard to escape.

The approach that we take, incidentally, to follow an allergen over a period of time is to actually do an immunoblot either with SDS gels or with isoelectric focusing and take a picture of it, and we have picture albums of all of our extracts every six months. Even though they're not done concurrently, because time is sort of that indisputable factor that you can't avoid, in fact you can follow how the allergens look from time to time by using the same serum each time.

It's not a quantitative bit of reassurance. It definitely is qualitative, but it's better than nothing, and that is something that we do find, and we do follow that.

DR. OWNBY: Jay, have you done what seems to me to be one of those obvious internal controls, and that is that you know your allergen extracts are pretty stable anywhere below 20 degrees, whether it's 4 degrees or -20 or whatever, in 50 percent glycerol? If you had aliquots of

the same extract and you lyophilized one and reconstituted it the next day to compare it, that seems to be a control over whether the lyophilization process is grossly affecting your extract.

DR. SLATER: That's exactly the study I described about four and a half hours ago. When I was telling you about the reference replacement program, one of the things that we're going to be doing is we're going to be taking a portion of every extract we get and we're going to be lyophilizing it, or we're going to be contracting to the manufacturers to lyophilize it for us, and then one of the things we're going to look at is what does that product look like if we reconstitute it immediately and compare it to the other product, and then over time. But it's a very important question, and I'll bet you anything that it's going to be a different answer for every allergen we look at. It's going to be completely different.

But obviously, to lyophilize a glycerin product, you have to dialyze out the glycerin. It's a big deal. But clearly, the question you're asking is a very important one that we're going to be interested in looking at.

DR. OWNBY: Okay. So it looks like on these issues we decided we want CBER to remain in the business, so to speak; that we think national standardizations are

very important, and that hopefully those can gradually be improved. You've shown us some of the incremental improvements in the enzyme inhibition assay and so on today. No question about the need for clinical testing.

The in-house lot release testing, I'm not sure, Tom, where you had a question or concern about that is --

DR. HOFFMAN: It's just in the interest of being complete. I mean, we expend huge amounts of effort testing lot to lot. The poles would be testing every lot or not testing at all and just accepting the manufacturer's data. You see that we test ourselves a certain percentage of lots, and that is a policy. Other parts of the center, particularly outside the Office of Vaccines, have dispensed with lot release testing. But we do it and we're committed to doing it. So I put it on the list because we've described our lot release testing program, and you see the amount of effort that goes into determining our limits for lot release.

DR. OWNBY: So you're doing this on a random basis?

DR. HOFFMAN: Random in your sense, Dr. Saxon.

DR. OWNBY: I mean, when the manufacturer submits data, they don't know whether this is one you're going to choose to validate or to do an in-house comparison of the data they've generated.

DR. HOFFMAN: That's largely correct. I mean, we do a lot of testing.

DR. OWNBY: And do the manufacturers have to pay for this?

DR. HOFFMAN: No. That's funded by the center.

DR. OWNBY: So that's part of your expense line, is to do this.

DR. HOFFMAN: Right. That's one reason I think that other parts of the center stopped doing it.

DR. SAXON: I think the way Jay described it really is providing a boundary assay, not how close are you to the mark. If you're really outside the boundary, it sounds like a good idea because these things can be variable, because it isn't a simple process. It seems like a very appropriate approach what you're doing, where Jay hasn't tried to tighten it up here. He's put the outer limits, so maybe 5 to 10 percent who really are outside shouldn't get released. But it won't become burdensome.

DR. HOFFMAN: I certainly don't mean to imply, by the way, that other parts of the center that aren't doing it are doing it willy-nilly. I mean, they have a reason.

DR. SAXON: No, I understand. But this is a complex material you're dealing with. As I say, it isn't so mundane, the materials.

DR. WRAY: I gather you must find some differences, some lots that don't meet the criteria, which is one reason you continue doing it. Is that a fair statement?

DR. HOFFMAN: That's correct. I think that Jay showed the percentage of out-of-spec lots over the course of a certain period. So some lots do fail. That's correct. Now, it is possible, just for completeness sake, that a manufacturer who has gone through lot release can request an exemption from lot release, and one can look on an individual basis. If a manufacturer has a long history of absence of failures, they themselves can apply and we could conceivably allow them not to submit lots any longer.

DR. OWNBY: It seems to me a very valuable function to keep everything honest. It makes the whole system more believable. Certainly you don't have to recheck every lot, but when there is the threat that a lot can be rechecked, then that should keep things more consistent. Obviously, it's up to a sampling statistician to decide what fraction of lots need to be done.

DR. EGAN: I'm Bill Egan, Acting Director for Office of Vaccines. I think, as was expressed, the lot release testing program is both a quality assurance and an insurance policy that we think is a core value, a core function for the office. In the quality control testing, I

think that there needs to be feedback loops, statistically determined feedback loops, and I think these have been in some sense brought into the process. Companies that have good compliance in their history of testing is less; those that don't, the amount of testing is increased.

If unusual things start to happen -- for example, it may be that for some particular product, there are a large number of failures from lot release. We may want to look more carefully at the ones that come in, and so on. But I think that there does need to be and is a statistical feedback loop in the process for governing how often we test, and which products.

I think we also need to keep in consideration the concept that if there are any problems with products, that we have the ability to explore those difficulties and problems, and if we give up our quality control testing and a problem occurs, the first thing we do is say, well, how do we do this? Is this really a problem? Unless you have these up and running in a validated and constant manner, you're not able to just simply delve in and try to repeat some lot release test. I think you fool yourself if you think you can.

DR. OWNBY: I think we've already had some discussion on some of the other issues on basic allergy and immunology and analytic characterization, the molecular

biology. I'm not sure. Does anyone want to comment, then, under Item 3, its regulatory role in terms of organization or with respect to manufacturers?

Betty?

DR. WRAY: I would just say that communication is key, because as we clinicians have problems getting extracts and complain to our sources, then sometimes they refer us back to this group, and this group has not met regularly, and I think it helps. It doesn't necessarily have to be face to face every time, but I just think having fairly frequent meetings so that those of us who are involved can provide some feedback to our colleagues will be helpful.

DR. HOFFMAN: I just want to respond to that. I think that's been a major effort. I think it's the culmination of an effort of about a year to try to make the communication, what you're pointing to, better with the committee, with the field, with the practitioners. I did go to the joint meeting in November and I found that very useful myself, and I think it made a difference in communicating. We also tried, to the degree that it's possible, without any conflict of interest, to speak to the manufacturers more and get some dialogue on just these issues, and to open up the issues of lot release testing and the approaches that we take so it's not inscrutable and

the basis for these decisions is shared with everyone.

That is, if we say the ninhydrin test, if we're considering something like that, to make it clear that we're not going to just maintain the ninhydrin test because it was maintained before, but at the same time we're not going to let go of any of the oversight unless there's a basis for it, and we invite your comment, their comments. So I think that's part of the goal of today.

DR. OWNBY: I would echo what Betty just said with respect to the medical community. I think we've had the perception at least that the FDA was acting in isolation and that perhaps one of the things we ought to work on is setting up appropriate forums at some of the national allergy meetings so that the average practitioner gets a better idea of exactly what's going on. I think even among the committee members, some of the questions today, you sense a certain naivete, and I don't think that many practitioners have much of an idea of which products are regulated in which way and how some of these were derived.

Even though some of that data was presented initially in a scientific forum, to go back over it and see it in its context of regulation of current extracts would be useful and important, and perhaps something that the members of this committee can help with.

DR. HOFFMAN: Well, that was the part that I was going to pick up on. Understandably, when we practice, or when we do anything, the nitty-gritty is often very uninteresting to anybody unless there is a problem, and some of these issues might be perceived as boring, rote, except when there is a problem. But we're also trying to reverse that impression, and by your explaining it when you go back to your community, by our participation at these various meetings and addressing the issues in the same way as we did here, we hope to keep an understanding going such that it's not only when there's a shortage or there's a problem or there's a perceived problem that the FDA activities garner attention.

I agree strongly that that's not the way it should be. Jay is going to the meeting in Orlando, and I think that will help disseminate the message. But I'll reiterate the fact that I'm available any time during the business day to field calls from members of the committee. If questions come up, you can have them call me, have them call Jay, have them call any of the staff. If they can't get through to compliance directly, we'll mediate calls to compliance.

In terms of education, I think it's a very important function that we're very willing to do because in the long run it makes our job easier.

DR. OWNBY: I was just noticing, how much of this is available or has been thought about in terms of being available on the World Wide Web? Because as these little crisis things come up, that there's a shortage of this or that, it seems to me that rather than you having to take the calls or Jay having to take the calls, if people knew that you could easily obtain that message somewhere on the Web, that it would be more effective that way.

MS. BRIDGEWATER: Hi. Jennifer Bridgewater, FDA. I can comment on that. We have put a lot of educational information out on the CBER Web site. When we entered the grass standardization process, copies of the letters that we sent to the manufacturers regarding standardization and dear doctor letters were available on the CBER Web site, and there were also some other informational items that went out that are available on that Web site, as well as major compliance actions. Those are also on the CBER Web site. Also, we meet with the AAAAI I think every other year. So we do have discussions with physicians about some of these issues.

DR. OWNBY: Do you know whether on the AAAAI or on the American College Web sites, if they're cross-linked into your Web site?

MS. BRIDGEWATER: Well, obviously, from the CBER Web site we don't cross-link to manufacturer or those

kind of organizational Web sites.

DR. OWNBY: I was thinking from the professional organizations to your Web site.

MS. BRIDGEWATER: I don't think so. I've been on the AAAAI and I don't recall seeing a link to the CBER Web site, but I'm not absolutely sure.

DR. OWNBY: I don't recall that either, but I haven't searched that diligently for it. But it seems to me that that's one of those areas that could be helpful because, obviously, as you try to come into government organizations on the Web, there are more than enough to spend your lifetime searching through them. But coming through some of the professional societies with links into your site might be the most effective way to make this more visible to people.

MS. BRIDGEWATER: That's a good suggestion, and I'm by no means a Web page expert, so I won't get into that, but those resources are available.

DR. HOFFMAN: The two aspects of the problem is, rather than the paucity of information, the overabundance of information. I think a lot of information is on the CBER Web site. All the actions that Ms. Conn alluded to are on the Web site, and copies of a lot of this information is on the Web site. What probably isn't there are division Web sites. Getting a Web site in the

government is not the same as putting it out in private industry. So there are very strict rules about that. If we had our own division Web site, we probably could enhance the communication even more, and I think we'll take under advisement to try to find a way to get some of the information that we've discussed here on the Web sooner and in a way that's more available.

DR. FREAS: I have been asked to remind the committee members, and the audience as well, that the transcripts from all our advisory committee meetings are on the Web site on the CBER home page and in advisory committees, and that generally occurs within 10 days after the meeting. So definitely you can follow up on any advisory committee issue on the Web page.

DR. OWNBY: Any further items or comments under discussion?

(No response.)

DR. OWNBY: I think that we can move on on our agenda to the closed session then.

DR. FREAS: Mr. Chairman, if we could take a short break before we go into the closed session.

First of all, I would like to thank the audience for their participation in today's meeting. Next of all, I would like to inform them that, unfortunately, the only people who can remain in the room after we come

back from the break will be committee members and FDA staffers with valid I.D. cards. We're asking everyone else to please leave during the break. All cameras must be turned off. All briefcases, purses, and other items must be removed from the room. Any briefcase or personal item left in the room will be placed on the FDA table outside of the room and you may claim it once we clear the room. Thank you for your cooperation.

(Whereupon, at 3:12 p.m., the open session was adjourned.)