PLANT-DERIVED BIOLOGICS MEETING

April 6, 2000, 8:00 a.m.

Scheman Building Auditorium

Ames, Iowa

Reported by: SueAnn Graham, CSR,

I N D E X

P R O C E E D I N G S

KATHRYN STEIN: While people are sitting down, I have a couple of announcements to make. First is that there are some handouts from talks yesterday and talks today that were not in your book, and they are available at the registration desk.

And secondly, there was a scarf found in the auditorium yesterday afternoon. It was one purchased in the museum store upstairs, and that is also behind the desk if it belongs to you.

This morning we have a program to hear some of the clinical experience for the products that you heard about yesterday. I think it's very important to put the manufacturing and environmental details in context and hear what experience there has been, and I think this will also set the stage for both discussion at the end of the session as well as the round table for regulatory issues that might arise or clinical issues.

Our first speaker this morning is Dr. Joseph Jilka. He has a doctorate in biochemistry from the University of Illinois at Champaign-Urbana. He has worked at Pioneer Hi-Bred, and he has worked at Monsanto Agricultural Company, and currently, he is the vice president for product development at ProdiGene. And his topic this morning is animal vaccines.

JOE JILKA: Thank you to the organizers for the invitation to speak about some of the animal feeding study that we have done at ProdiGene. ProdiGene actually grew out of Pioneer Hi-Bred, and we're now located in Southeast Texas at College Station, Texas, the home of A&M University.

We have a number of different interests.

We have an interest in food additives. The area that I'd like to talk about today is our commitment in the area of animal health. Well, why edible vaccines? Perhaps I'm preaching to the converted crowd, but I think the edible vaccines allow us to explore a whole new area of animal care, vaccinations without injections. We can deliver the vaccine as part of the feed. We can really explore the feed as a new tool for animal health-care management.

And we do know now from our work that the edible vaccines will induce both a mucosal and a systemic response. However, the jury is still out on whether we really get cell-mediated immunity. There’s a lot that we can do with edible vaccines and they can be used in combination with conventional vaccines.

Why edible vaccines in corn? Well, we do know that recombinant proteins in corn express well. They fold correctly. We get great activity out of them. The glycosylation patterns are at least similar, although as we all know, they're not exact. And we do know from a number of studies that we can store the seed at ambient temperatures, and conditions that simulate, for example the Iowa summer, and hauling around in grain cars and storage in bins, without degrading the recombinant proteins. As Carole Cramer said yesterday, they're nature's own storage device.

Also, the seed itself is of very low cost. It costs $2.50 to produce, although on the way up here, I heard the selling price was only $2.19. Also there's a vast infrastructure for growing, handling, and processing grain. We don't have to reinvent the wheel, so we can take advantage of what's already out there for processing. And of course, the seed itself can be ground or used directly in the food and the feed.

Well, I'd like to focus in particular on one of our animal vaccines which is our leading candidate; the one that we're advancing the furthest for our animal health-care products, and that's a vaccine to the swine transmissible gastroenteritis virus. This is a product that's been percolating for quite a while. It actually started out at Pioneer in a collaboration with Diamond Animal Health. Ambico was actually working on this at the same time. Prodigene took on this project.

As one goes through the work on the development of a product there are certain landmark pieces of data that really stand out and that make you feel like, "Yeah, this is really going to work."

When we first started out at A&M, we had a lot of Avidin corn on hand with really pretty good detection assays that we could work with. We began to do mouse feeding studies to explore the whole concept of edible vaccines.

These were done in conjunction with Dr. Ian Tizzard at the College of Veterinary Medicine at A&M. If you've come up through the veterinary ranks, you've probably grown up on his immunology text book.

These are mice that have been fed avidin corn and this is just the control. These are western blots. This is the avidin that has been isolated from corn. These are aqueous fecal extracts of mice that have been fed avidin that's been spiked into mouse chow vs. avidin expressed in corn. It looks like the purified naked protein has been destroyed in the gut. However, contrary to earlier worries with edible vaccines, when you feed the mice avidin that's expressed in the corn, we actually do see avidin that's being picked up in the fecal extracts. This lets us know that there's Avidin encapsulated by the corn that can essentially slowly bathe the entire gut surface. Antigen can leach out allowing us to reach the entire length of the gut.

Now, to focus on TGEV. As we all know, it's an envelope coronavirus. We chose to express three of the major proteins in corn, the spike protein, the matrix protein, and the nucleoprotein. We expressed these separately in individual lines.

Because these are viral proteins, we resynthesized the genes for maize high codon usage. To our pleasant surprise, when we began to get those initial lines of corn back, it looked like we were getting levels about 400 times higher than what had been seen previously by us or in the literature.

Some of these initial lines were as high as 0.8 percent total soluble protein. Divide that by 100, and that gives you 0.008 percent by dry weight. For initial T1-seed this was quite high. This gave us more confidence that this technology could actually work.

As we began to get some initial grain back from those early events, we sent the high expressing events off into a breeding program to get them into elite germ plasm. We also took some of that grain to begin some initial feeding studies.

This was a study that we did in conjunction with Bruce Longhorn at the Vet school, at Texas A&M. We looked at twelve piglets total. There were four treatments in this study group, and there were three pigs per treatment.

We fed them 50 grams of corn that was expressing the spike protein for 14 days, and then on Day 22 we challenged them with TGE virus. We collected fecal samples every other day, and we collected blood samples weekly.

Now, here's the results of that study. You'll notice there's only three treatments up here. The fourth group was fed LT-B corn, which expressed the B subunit of the E. coli enterotoxin.

We included that in the study as a potential marker, or in case the TGEV didn't work, at least the LT-B might. It turned out these pigs had a history of E. coli infection, so we threw that out of the study.

If you look at these three groups, here is our challenge data. We originally had the virus titrated to see really good clinical symptoms at Day 15. We wanted to see if we could follow the IgA and IgG responses.

It turned out that our analytical tools weren't really sensitive enough to do this, but we put all the blood samples through a virus neutralization assay. Also, we delayed our challenge one week, and the piglets were, therefore, the less susceptible to infection.

It actually turned out very well in that we were able to induce a subclinical infection of these piglets, and it appeared that the pigs that were fed S corn had a very strong perhaps anamnestic response to this protein.

At any rate, it showed that for the first time in our hands the immune system of these pigs could actually see the S protein in the corn and react to it.

Based on some earlier avidin work, we know that by varying the timing of the presentation, whether it's seven days on, seven days off or continuous or three days on, three days off, one can profoundly influence the relative degree of IgA and IgG responses.

In a second TGEV study, we again fed pigs 50 grams of S corn for seven days, and then regular feed for seven days, and then seven days on TGEV corn again and then seven days off again. We had hoped to see a pickup in response on the second feeding. We then ended up challenging them with a weak challenge on Day 29.

We essentially got the same result as before. Here is the day of challenge, and then here is the very strong response. This is an even stronger response than in the previous study. Perhaps the seven days on, seven days off a regime gave us a better immune response. However, it could be just due to our neutralization assays, not being sensitive enough. It also could be that what we're getting is primarily an IgA response instead of an IgG one. At least in the neutralization assay, you can't really see any primary response. But when you challenge them, you certainly see that very strong response in the pigs that previously saw the S protein

This really gave us a lot of confidence that we could put an antigen in corn, and that when pigs consumed it, their immune systems would detect it.

Now, this is a trial that was done in conjunction with Dick Hesse. At Intervet over in Dallas Center. This is part of the data. What we wanted to do in this study was to examine sows.

There were three treatments in this study and five sows per treatment group. The treatment groups were control corn, S corn, and Intervet's modified live vaccine.

We fed them way out here five weeks prior to farrowing and then took blood samples or serum samples and ran those through the neutralization assay.

With a live modified vaccine, as you would expect, we saw a very nice serum response, but at least prior to this data point, there was no response with the S corn.

This is the date of farrowing. We also checked the colostrum and with the pigs fed the S corn we didn't see any appreciable level. So this was kind of a bummer for us. Again, a really nice response with the modified live vaccine.

Then, two weeks post-farrowing, we gave all the treatment groups an injection of the modified live virus, essentially to stimulate a challenge.

Then, the response with TGEV corn began to rise. So I think this strategy is going to work. I think it's just a matter of how and when. Perhaps we need to feed a little bit later rather than five weeks before. This indicates that there's a lot of parameters that we have to explore yet.

In conclusion, we do know from these studies that piglets and, I think sows , can react with an anamnestic response when they are fed an antigen expressed in corn seed. They do appear to be primed when they're exposed to the virus.

The most extensive study then was done just earlier this year up here in Iowa with Mark Welter of Oragen Technologies, and this was done with three treatment groups with10 pigs per treatment. These are 10- to 12-day-old piglets.

Again, we fed them 50 grams of the S expressing corn for 10 days, and then on Day 12 we challenged them with virulent virus which had been titered for greatest clinical symptoms on Day 12. Then the clinical symptoms were monitored for two weeks.

The clinical observations were divided up in three different ways; a morbidity incidence, a morbidity incidence and duration, and a clinical severity index.

We look first at the total clinical symptoms that are based on a morbidity incidence, which is basically how many pigs had clinical symptoms. Our clinical symptoms were, of course, those characteristic of TGEV virus and were based on a certain score of four if the pigs had watery diarrhea and then reduced scores for other types of symptoms.

. This is how the data looks. In our control corn group, we had great symptoms. Happily for us, at least on a morbidity incidence, about half of those piglets fed TGEVC corn never developed any symptoms at all. We saw a bit more evidence of disease in the modified live vaccine positive control group. I should say that there were three treatment groups in this study: the control corn, the S corn, and a modified live vaccine.

Now, if you include a time facto it's interesting that the symptoms with the modified live vaccine clear up faster than with the S corn

It's really interesting, I think, and it might point out that this delivery technology ws, a different mechanism of immune response and protection to that seen with a live vaccine.

Now, if we express these data as disease severity index, we again see clear symptoms with our control corn, and now with the S corn and the modified live control, we see protection.

In the interest of time, there's one slide that. I did drop out but which is in the handout. We are looking into the poultry market, and in the handout there's a picture of the response when LT-B corn is fed to chickens. Their immune system will react to the LT-B, and you get an immune response, at least in serum.

In conclusion, we do think, as I'm sure everybody else does here, that the production of proteins from plants will give us products that are safer, and are easier, and cost a lot less to produce.

In particular, transgenic plants will give us a whole new system to deliver vaccines and perhaps even growth promoters, protein-based antibiotics, and all kinds of different things to animals through the feed. This is going to be a whole new way of performing animal health care.

Edible vaccines in corn will give us stability during storage and hauling, as well as ease of processing. You can haul them around. Ease of processing, perhaps through milling.

We can take advantage of the low-cost production system that's out there. There are no capital requirements for new fermentors. You essentially use the farmers' capital system.

In particular for TGEV, we see a high level of expression, and we do now know that these antigens, when they're expressed in corn and fed to pigs will protect the pigs from a viral challenge. Thank you.

KATHRYN STEIN: I think we do have some time for questions. We're a little bit early, so hang around. Are there some questions for Dr. Jilka? Yes. First to the microphone wins.

PATRICIA SHEWEN: Thanks. Pat Shewen, University of Guelph.

KATHRYN STEIN: Is that on?

PATRICIA SHEWEN: Okay. Better? Pat Shewen, University of Guelph. Two questions. One relates to that last comparison you did with the modified live viruses, and I'm suggesting – or based on comparisons between live and killed viral vaccines that the modified

live is giving you -- or can induce a cytotoxic T cell response whereas naked antigens won't do that and whether you've looked at that contrast at all in these trials.

And the second question kind of arises from that in that in the absence of a cytotoxin T cell response, animals often become carriers. So how have you looked at whether your vaccines in animals are carriers of this challenge?

JILKA: In response to the first questions, no, we haven't really gotten into the cellular aspects of how this immunity works.

PATRICIA SHEWEN: I would be interested to know how you would postulate that you could get that kind of a response from feeding them.

JOE JILKA: I'm sorry. What?

PATRICIA SHEWEN: I'm asking whether you have considered how you might induce that response when feeding an antigen that's not a live organism.

JOE JILKA: Yeah. Essentially it's a subunit vaccine. We do have the matrix protein that we have yet to bring along. I don't know. That's a good question. I think that whole area has to be really worked out yet.

PATRICIA SHEWEN: And have you checked to see whether you're creating a carrier state by vaccinating the way you do?

JOE JILKA: No, we didn't. We checked them at time of challenge to make sure hat the virus came through; that the clinical symptoms were due to the virus challenge, but we didn't check them, at the end of the study.

PATRICIA SHEWEN: So --

JOE JILKA: But it would be a good thing to check.

PATRICIA SHEWEN: That's important if you're looking at disease dynamics.

JOE JILKA: Sure.

PATRICIA SHEWEN: And actually, I think of one other thing. Right at the very beginning when you were showing Avidin, the presence of Avidin in feces --

JOE JILKA: Right.

PATRICIA SHEWEN: -- did you have undigested kernels in those feces?

JOE JILKA: Right, yeah.

HILARY KOPROWSKI: Is the microphone on, sir?

JOE JILKA: Yeah, that's true, and some of that could have been leaching out from undigested particles. But the point is if it's leaching out there, then it could have been leaching anywhere upstream.

PATRICIA SHEWEN: Well, that was going to be my next question. Did you see anything to see if, in fact, it was leaching or if it was contained within those undigested kernels?

JOE JILKA: Oh, I see. No. It definitely leaches out.

PATRICIA SHEWEN: Thanks.

KATHRYN STEIN: The other side of the room.

WALTER GOLDSTEIN: Hi. Walter Goldstein with Biolex. Just a question on the response. When you feed the vaccine on the corn and it passes, say, in the intestine, there's a certain residence time and passage of it in the intestine that could be affected by the way you're feeding it but whether you're giving it in pulse or continuous or duration. And I wonder if there's any relationship to that to the immune response in the animal.

JOE JILKA: I guess we don't know. If you make a matrix of things, there's a lot of things to do. Is it timing? Is it particle size? How fine are the particles that we feed to the piglets? I think there's just a lot of work to be done yet.

WALTER GOLDSTEIN: Thank you.

SURINDER CHOPRA: First question is what did the promotor use for this transgenic TGEV-S.corn?

JOE JILKA: These were research lines that were done with a ubiquitous type promotor. However, all of our new product-type lines that were really going to product with are seed specific, and they're giving about as good a level of expression as the ubiquitous type.

SURINDER CHOPRA: Second question is you mentioned you have created transgenic line with all three proteins. Do you need to cross all three to get the work or just S, or just what in the protein already gave you the effect?

JOE JILKA: We can easily transform all of them into the same plant, and express them together. Right now they're in two different lines If we need to we'll just blend these together for the treatment --

SURINDER CHOPRA: Okay.

JOE JILKA: -- right now.

SURINDER CHOPRA: For the spike experiment you have done, that's 50 grams of corn.

JOE JILKA: Right.

SURINDER CHOPRA: Can you tell us what amount of S protein or anything?

JOE JILKA: Oh, sure. That had about -- oh, I should have done my math, but I think it was around a milligram or so of S protein in there.

SURINDER CHOPRA: Just spiked extracted from the corn and spiked into the corn?

JOE JILKA: No. If you check the levels of S protein in that 50 grams of corn, there was about a milligram of S protein in there.

SURINDER CHOPRA: Thank you.

MADAM REPORTER: Excuse me, ma'am? Could I get your name, please?

SURINDER CHOPRA: Chopra, Iowa State University.

KATHRYN STEIN: We have time for one last quick question.

JENNIFER CONLON: Jennifer Conlon from Merial. I found the duration of your experiment very short, and I was wondering if you had any information on duration of protection or duration of any kind of response because with a killed antigen, you would postulate that the protection was associated with antibody, and you would probably need relatively high levels of antibody to block any kind of infectivity, especially with a virus.

And your challenges are very short, and even your detection of antibody responses were very short lengths of time. Have you done anything to show duration of antibody how the antibody levels are dropping?

JOE JILKA: No. I understand exactly what you're saying. We're planning on doing those experiments. But we haven't done anything like that yet.

JENNIFER CONLON: Okay. Thanks.

KATHRYN STEIN: Thank you very much.

We'll have time for more questions at the end, all of the speakers in the session.

Our second speaker this morning is Dr. Hugh Mason. He has a Ph.D. in cellular and developmental biology from the University of Arizona. He did post-doctoral work at Texas A&M where he began studies with Charles Arntzen in the plant biotechnology program, and currently, he's working in Dr. Arntzen's group at the Boyce Thompson Institute for plant research at Cornell University. He's going to talk to us about edible vaccines for human use.

HUGH MASON: Thank you. Thanks very much to the organizers for inviting me. It's a pleasure to be here.

So I'm going to talk today about our experience with three different candidate vaccines. The slide shows E. coli, LT-B, and Norwalk virus are candidate, vaccines for the diarrheal disease.

And the other one is the Hepatitis B vaccine, which is the first one that we began working on back in 1991. Actually, we showed the expression of the surface antigen in tobacco.

The diarrheal vaccines were tested at the Center for Vaccine Development in Baltimore. That was funded by NIH. Also want to thank NIH for funding a collaborative grant with that group and with John Clements at Tulane and Mary Estes at Baylor in Houston to develop multicomponent diarrheal vaccines that led to this work in clinic. And the Hepatitis B trial was a boosting trial. It was performed at Roswell Park Cancer Institute in Buffalo, New York. Yasmin Thanavala was the project leader on that. The funding for that study was the Roswell Park Cancer Institute. Also, Axis Genetics in England, a company which has since gone into bankruptcy, but hopefully that is not a grim foreboding for the plant vaccine efforts.

But just judging from the enthusiasm and the amount of work that's going on in this area, I think that certainly we will be able to develop it sufficiently that it will be applicable in the near future.

All of our studies were done with raw potato with the antigen expressed in the potato tuber. And you can see preparation of the tubers.

There's Dwayne Kirk who's our project coordinator, preparing some potato tubers at the Center for Vaccine Development, and this was for the Norwalk trial. All of the studies were using an IND permission obtained from FDA. These were double-blind studies designed with

the evaluation of serum and fecal antibody production. There were a total of 82 human volunteers in all of the studies.

Before I go too far, I want to just acknowledge some people that were very important in all of these studies. Linda Rosendorf at NIH for her support in the development of the INDs, and without her help I think the first two studies certainly would have never got off the ground.

Of course, Charlie Arntzen, my co-PI on these projects to develop the expression of the antigens in the potatoes. A host of post-docs but mainly Liz Richter who worked on the Hepatitis B work; Tariq Haq, a graduate student at Texas A&M who did a lot of the LT-B work, and Bryan Maloney especially, a technician at BTI who participated in a lot of the Norwalk virus work. And our collaborators, John Clements and Mary Estes Imentioned already. Carol Tacket and Myron Levine at Center for Vaccine Development who were the project leaders at the clinical trials in Baltimore and, of course, Yasmin Thanavala at Roswell Park. I just want to use this slide to illustrate the mechanism of antigen delivery in the gut. If we blow up the human intestine, as unsavory as that sounds, but many of you are quite familiar with the anatomy of the lymphoid tissue there, but there are enterocytes lining the gut here, and these areas are called Peyer's patches where lymphoid cells aggregate and reside.

There are specialized cells called M cells which are shown here that participate in active sampling of the antigens and transfer across the mucosal barrier to allow antigen presentation to the lymphocytes that underlie.

And it's a very complex cross-talk that occurs between these cells in order to stimulate the antibody production and response to the antigens.

The M cells, I should point out, appear to be somewhat specialized for the uptake of particulate antigens. In other words, they have a preference for antigens that are particulate in nature such as virus particles or even up to the size of bacterial cells. But an antigen like LT-B, even though it's a very small antigen, is apparently actively sampled in this tissue. So I'm going to talk first about the LT-B trial, the first clinical trial which occurred late in 1997. Liz Richter showed you a slide yesterday that illustrated the antigen. The B subunit shown in red here is actually a pentamer of monomers that when assembled is able to bind GM-1 gangliocides that are displayed on the surface of eukaryotic cells.

And this protein is very similar to Cholera toxin B subunit, and CT-B had been used previously in clinical trials and was determined to have some benefit in the Cholera vaccine by virtue of the fact it could interfere with toxin binding and therefore interfere with the delivery of the toxic A subunit. So we showed initially that the LT-B subunit could be expressed in tobacco and potato, and Liz showed a slide yesterday on preclinical results such that feeding the raw potatoes to mice would induce both serum IgG and fecal IgA directed against the LT-B. Our clinical trial -- before I go into that, I want to illustrate we had to begin thinking about how we would present our preparation or the manufacturing practice to convince people that we had

a uniform way of processing the material. This slide just illustrates that we start out by transforming the potato with Agrobacterium, develop transgenic lines. These lines. Once they're isolated and propagated by vegetative clonal propagation, stem cuttings in vitro constitute a master plant bank which can at any time be cloned to develop as many individual plants that are genetically identical as we would like.

These plants are transplanted in soil, and in this case we grew a Generation 1 plant to yield tubers which were then used in seed to produce Generation 2 tubers which were then used as the vaccine. So this is the scheme we used for the LT-B. For Norwalk we actually went directly to the vaccine trial in Generation 1 material. So the LT-B trial, again, was with Carol Tacket as the principal investigator. John Clements, of course, was an active participant. John performed all the preclinical work at Tulane. So you can see here that preparing the samples was a bit more arduous than the normal vaccine preparation where you're simply taking a bottle and injecting the vaccine. You can see the nurse doesn't look all that happy about it. It's the first time that she's ever done this kind of vaccine preparation, I'm sure.

And although the whole plant vaccine system is supposed to develop a more convenient delivery system, at least in the early stages, I think we can all agree that it's perhaps not nearly as convenient as an injectable vaccine.

In any event, there were 14 volunteers who ate the transgenic potatoes, either 50 or 100 grams of potatoes in three doses at Day 0, 7, 21.

There was a substantial variability in the antigen content in these tubers. However, 100-gram dose delivered approximately, I think, around 700 micrograms of LT-B. There were no significant adverse side effects, although there were a few complaints of stomach upset or maybe one complaint of mild diarrhea. In one case it was with a control nontransgenic potato. I don't know how many of you have tried to eat raw potato before, but some people tolerate it well, and others tolerate it perhaps less well. But in general the side effects were not too significant. And it's possible that some of the stomach upset was due to the proteinase inhibitor that are present in potato tubers, and it's more nthan likely that the presence of the proteinase inhibitor could have contributed to the stability of the antigen as it passed through the stomach. But again, we don't know that for certain. We have no data to support that.

Carol's group at CVD took peripheral blood,- mononuclear cells, and looked for antibody secreting cells that were either IgA or IgG specific. The orange bar shows IgA, and the blue are IgG specific antibody secreting cells. The doses, again, were at zero, seven, and twenty-one days, and these are geometric mean numbers from all of the responders. There were ten of eleven of the group who had the transgenic tubers that responded with increased levels of antibody secreting cells, and these are fairly significant numbers. With the IgA secreting cells, there was a mean of 18. Interestingly, the numbers went down at Day 14 even though there was a second dose at Day 7, and they went down almost to basal level at

Day 21 when the third dose was given, but the level of antibody secreting cells went up again at Day 28.

Carol also looked at the level of neutralizing antibody. Actually, this was a study done by John Clements, examined the serum IgG for neutralizing antibody. The profile shown here, geometric mean for the responders, ten of eleven responders, goes up progressively to Day 7 and Day 14. And then there was a dip between Day 14 and Day 21. And then after a third dose, it peaked again.

And the last data point at Day 59 had gone down, but still, there was a substantial level of neutralizing antibody in the serum of the volunteers. This study was published in Nature Medicine May 1, 1998. So in summary, ten of eleven of the volunteers responded with serum IgG against LT-B. None of the control placebo potato eaters developed any antibody Serum IgA against LT-B: six of eleven volunteers were shown to have serum IgA, and it's not indicated in this slide, but five of those were shown to have secretory IgA in their fecal samples. Relatively low titer, but realize also that the assay for fecal IgA is somewhat insensitive.

Also wanted to point out that this response was roughly similar to a volunteer challenge with virulent enterotoxigenic E. coli, Of course, we had no response against surface antigens of the bacteria.

And that's another point. It's likely that antibodies against surface antigens or colonization factors of the bacteria may be important in developing a good resistance to enterotoxic E. coli disease.

Okay. I'm going to go on now to Norwalk virus. This is a Calicivirus that's the major cause of nonbacterial gastroenteritis and very common. Probably all of us have had it very early in our lives. We decided to work on this with Mary Estes because it has a very simple capsid structure, a single capsid protein which when expressed in a baculovirus virus infected insect cell system is similar to a viral particle which has a diameter of about 38 nanometers. Mary had tested this in human volunteers. Actually, she had not done it in humans at the time that we began our study but in later studies indicated that the delivery by drinking 250 micrograms of virus-like particle could stimulate seroconversion or increases in serum IgG in five out of five volunteers.

So we did some preclinical studies. This shows virus-like particles produced in tobacco leaves, and this was published in 1996. And some data on gavaging the tobacco leaf material, partially purified, into mice either with or without CT showing increases in serum IgG against Norwalk virus. So in mice we demonstrated that our plant-derived material was appropriately immunogenic and appeared to be in all respects very similar to antigen from the baculovirus system. So we went on and developed another IND for this clinical study, again, at Center for Vaccine Development. This is Carol Tacket's group at Center for Vaccine Development. In this case we had twenty-four volunteers with two experimental groups. We wanted to try two

different dosing regimens. Again, the potato tubers were consumed raw and prepared in the same way, although this time we did a little bit differently in that we created batches by putting the potato tubes into a bucket of ice water and holding them until the doses (that were 150 grams of tuber material in this case) were doled out to the volunteers, and we saved a little bit of this material and froze it and sent it back to BTI for antigen analysis.

The antigen levels were quite variable, and that's one of the problems. In this case we were using the patatin tuber-specific promotor to drive the expression of the Norwalk virus capsid protein, and it turns out that the 35S promotor probably would have been better. Now we have more new lines of potato. We're using 35S promotors that do give us substantially higher levels of expression. And it's not clear why that is, but in any event, I wanted to mention that we did get extensive variability in levels of antigen that appeared to have some relation to the tuber age with the young developing tuber versus an older tuber.

The study was designed for three dosing regimes. Volunteers got 150 grams of the transgenic tubers on Days 0, 7, and 21, similar to the LT-B study, or only on Day 0 and 21 with a placebo on Day 7. And the last group got only placebo potatoes, at all times.

The data is here. In this study that is due to be published very soon, at least in the next couple of months, I think, 19 out of 20 volunteers who ate the transgenic potatoes did develop antibody secreting cells, in this case showing IgA specific antibody secreting cells from the peripheral blood. There's no significant difference whether the volunteers got two or three doses in any of these really. The responses were fairly variable. Again, antibody secreting cells varied between 6 and 280 for the two doses or between 6 and 245 for three doses. The stool IgA, we only had two of ten of the two-dose group or four of ten of the three-dose group responding. IgG responses were again variable. We had four out of ten volunteers for the two-dose group or two of ten for the three-dose group that developed serum IgG antibody secreting cells.

There was a fairly low geometric mean. Thirty-four antibody secreting cells versus 103 between the two and three-dose group. So there was no really good systematic difference that we could ascribe to the dosage levels.

The levels of variability are somewhat disarming but probably relate to the variability in the antigen in the tubers which we couldn't really control very well but also to the high variability of the population of the volunteers and the likelihood that some of them could have had a Norwalk infection more recently than others. So the summary then just shows 19 of 20 volunteers had some kind of immune response and that there were IgG, IgA, and IGM specific responses determined in some of the volunteers. Some showed a very robust response, and again, we don't know why. It could have been likely that it was a boosting effect, but we don't know for sure. And I wanted to point out also that I failed to mention that in our potato material only at most 50 percent of the antigen was assembled in the virus-like particles as determined by rate zonal sedimentation.

So we believe that if we can get both higher levels of expression and a better efficiency of assembly of this material into virus-like particles, it's likely to have a better stability going through the stomach and also be more immunogenic once it reaches the effector sites in the bowels.

So our future studies are already doing that. We want to do hopefully this year a clinical trial in which we will do a challenge with virulent Norwalk virus. Luckily, Norwalk virus is not a very severe disease. It's debilitating for about a day, but it lasts only about a day or two, and as long as we have volunteers in the hospital, it's reasonable to do a challenge study there. Finally, I want to go on to the talk about our Hepatitis B work. We early on showed we could express this material in the form of a virus-like particle in tobacco. There were a number of reasons for wanting to use plant vaccine that have been gone over by others. Preclinical work done by Yasmin Thanavala, and Liz showed a slide yesterday that indicated the data in red here where Yasmin fed the potato three weekly doses and then challenged with a single i.p dose and showed after the transitory primary response a very strong anamnestic response.

She showed also the data in blue that you could prime with a single injection and then actually boost by feeding potato tubers to the mice at a later time.

Our clinical study that was done at Roswell Park recently was designed this way. We recruited volunteers who were health-care workers that were vaccinated over five years ago and who had at the time of the study low anti-HBV titers. We wanted to feed them the HB tubers, 100 grams per dose, approximately 1 milligram of antigen, and look at the boosting responses. So there were three doses, seventeen people fed at zero, two, and four weeks, and another group had two doses at zero and four weeks. And as much as I would love to tell you about the data, Yasmin would like us to remain silent about it until we get it published or at least accepted for publication.

The manuscript is in process at the moment, and we're hoping to get it ready for submission in the next couple of weeks. So again, I refer you to Yasmin, and I'll let her tell you as much as she'd like. But we think that certainly it will be easily publishable.

My last slide just shows the conclusions. There were no serious side effects observed with the use of transgenic potatoes in humans or in animals. And it appears that encapsulation of the material in the plant cells was sufficient at least for some degree of antigen presentation and protection. And both systemic and mucosal IgA and IgG responses were observed. In the future, as Liz mentioned yesterday, we're working on tomato, and we're also working on banana, and we're hoping to go into legumes in the future. We want to obviously work on ways to increase levels of antigen expression and the assembly of the material into appropriately antigenic forms. We also want to work on post-harvest processing and delivery because we think it's going to be very important due to the variability that we've observed to have a material

that's processed to some extent to allow us to be able to accurately deliver a very precise amount of antigen in each dose.

And of course, we want to look at the optimal dosing regimes, the amount of antigen and the frequency of the dosage that's needed, and examine perhaps the need or the inclusion of adjuvants in multicomponent delivery.

And finally, we want to examine the mechanisms of these responses, and Yasmin is involved in doing that presently in the mouse system. But certainly in human volunteers, we need to evaluate the mechanism of the responses as well. And I'll stop there. And I think I'm a little long, so reserve questions for later, but thank you for your attention.

KATHRYN STEIN: Thank you very much. I think we'll hold questions. If we stay on time, we'll have 15 minutes for questions for all the speakers at the end. So I'd like to move on to our last speaker in this session, Dr. Julien Ma. He has a degree in dentistry from Guy's Hospital where he also earned a Ph.D. in immunology. He then went to SPRITZ? Institute for post-doctoral work in Andrew Hyatt's group where he became involved in studies of the expression of recombinant antibody in transgenic plants.

He's currently a senior lecturer and consultant in immunology and oral immunotherapy at Guy's Hospital.

And he's going to talk to us about monoclonal antibodies produced in plants. Dr. Ma.

JULIEN MA: Thank you. Well, I'd like to thank the organizing committee for inviting me to this conference too. It's been excellent so far.

I've been asked to talk about our clinical experience with expressing monoclonal antibodies for topical immunotherapy in human volunteers. This is our teeth model. It's dental caries. And while it's hard to pretend that this caused a lot of mortality around the world, I think anyone who's afraid of dentists and certainly afraid of injections will welcome the kind of work we're trying to do. It's not a particularly common disease in animals either, although I did spend the first two years of my Ph.D. making sandwiches for our experimental monkeys and brushing their teeth every day. But I do believe that domestic animals if pampered are susceptible to this disease, but I can't imagine there's an enormous market.

There is, however, an enormous market in human health care in this area, and dental caries probably is the second-most prevalent disease on the global scale, second only to periodontal disease.

Now, tooth decay is a bacterial infection, and it's caused by this bacteria Streptococcus mutans, a gram positive coccus which is presently in about 50 percent of the population at any one time and when it reaches certain levels causes disease by secretion of assays which

demineralizes the tooth surface. Now, one of the important virulent factors resides in this fuzzy coat, which is on the surface of the bacteria, and this is a protein that we identified back in the '70s and turned an antigen one to. It's a NUCLEOPROTEIN, and it allows the Streptococcus to adhere directly to the tooth surface via a specific LYPAND? which is derived from the saliva which attaches directly onto the teeth.

Now, Streptococcal mutans II is actually a member of the family called the CACERN? proteins which are also found in other oral Streptococci. And I should tell you there are about probably 300 different bacterial species in your mouth and many, many oral bacteria of the streptococcal group.

But there is a fine specificity then within the prime immunoassay sequence which determines whether these bugs will attach to the teeth or attach to the tongue or the cheek or other environmental surfaces in the mouth. Now, to begin with, we were investigating ways of inducing active immunity against Strep mutans, and we titered the antigen for immune response. Now, working the mouth has a number of advantages if you're interested in mucosal diseases and probably is one of the more appealing mucosal surfaces to work in. I seem to have lost control of this. Could you go back one, please? Thank you. From an immunologist point of view, actually, one of the most interesting things is that it is at the interface of the serum immune response which arrives in the mouth through the space between the gum and the tooth, the tooth gingival crevice, and you get a lot of serum seeping out of here, which is an active exudate and contains immunoglobulins derived from serum, also the proteins and neutrophils.

But by far the greatest source of immunoglobulin in the mouth comes from secretions, and that's part of the mucosal system that we've heard about already. And we bathe the mouth in about a liter of saliva, and a major component of saliva, of course, is salivary antibodies. So there are two major sources of immunocomponents, and we have looked at stimulating antibodies in both ways. Now, we did actually come up with a vaccine back in the early '80s, but when we approached the regulatory authorities in the U.K., they really turned us down because the feeling was that dental caries is not a life-threatening disease, and they would want an absolute safety guarantee for any vaccine that was put on the market, which of course we couldn't provide. And this is really why we got into the area of passive immunotherapy because we felt that this would be a much safer approach.

So instead, we've made an antibody which is specific for Streptococcal Antigen 1-2. It's one of a panel of antibodies that we have, and we do have data from a number of these monoclonal antibodies, but I'm going to focus on this one, Guy's 13.

It was derived by conventional techniques from MANODERMITS?, and it recognizes a region within the Antigen 1-2 which maps to the adhesion determinant of this protein. And we've done a number of studies in human volunteers – or I should say dental students -- using this monoclonal antibody derived from mouse ascites, and those were done several years ago. I won't go into those again. And in all our past studies, our antibodies are applied directly to the

teeth. Again, this is another advantage of working in the mouth. Of course, one can think of many other delivery methods: mouthwash, toothpaste, whatever. But for all of our studies, we simply use this, and we use antibodies at a rather high concentration.

Of course, the first thing that people do when they put these things in their mouth is swallow, which is a bit annoying. And so we use antibodies at relatively high concentrations, 10 mgs per ml, and this turns out to be one of the reasons we feel that we need to go into plants because of course, if this product were to come into the market at current concentrations, we would probably need thousands of kilograms of antibodies per year just to satisfy the U.K. market for this product, let alone the U.S. market.

So our idea is that the antibody will adhere to the tooth surface or the salivary glycoprotein layer and compete with the natural receptor for Strep mutans for binding to Strep mutans. Now, we're not quite sure what happens at this stage, whether it is simply a blocking effect or whether there is some functions going on: bacterial killing, complement activation, or simply an aggregation. We have some ideas but haven't saw the data yet. I'm not going to tell you about the work with the IgG version of this antibody because all of our original studies using mouse ascites was done with the IgG.

But when we went into plants, we rapidly realized that we had the capability of making a secretory antibody version of this simple antibody engineering. And of course, we felt that there were a number of advantages to using secreting antibodies in the mouth. After all, this is nature's answer to protecting these mucosal surfaces.

Just to run over the structure of the secretory antibody which is a little bit more complex than the standard serum IgG antibody, you'll see that it's made up of two of these monomeric units, heavy and light chain, which are DITHERIZED? to a third protein which is called adjoining chain. Now, this immediately gives the advantage of an increased formidity for your antibody and also allows better aggregation of bacteria if that is indeed one of the protective mechanisms that's important. The fourth protein involved is secretory component which is normally made outside the plasma cell or epithelial cells, and this wraps around the dimeric structure. And one of the roles of secretory component in vivo is to protect this antibody against proteolytic degradation. And that, of course, is another advantage when you're using these antibodies in the rather hostile environment of the mouth. And for others, the gastric tract.

Well, we went about making secretory antibody of Guy's 13 in plants, and we did it rather a low-winded route by making four separate transgenic plants and going through a series of cross-fertilization to accumulate all the immunoglobulin subunits in the offspring. Some of the people often ask me whether I'd do the same again. The answer is I wouldn't, but I'd ask my post-doc to because I think we have also tried putting all four components into the plants at the same time or putting two and two. I mean I think there are time advantages to this, but one of the big advantages going through this system is that at each stage, of course, we allow ourselves

the luxury of selection of the best expressors, and I think that is one of the reasons why we get such great expression and accumulation in the final plant.

This is a western blot to show the assembly of these immunoglobulin units in the transgenic plants, and this is done on a 4 percent SDS gel and plotted. On this side we've taken an anti-light chain which shows up in all of the immunoglobulin assembly units, and on this side we detect this anti-secretory component.

And here you see the monomeric IgA, and then when you cross this part of the J chain, you see a bit of monomer, but you also see the diamer there. And then when you cross it with the secretory component, you get a monomer, a dimer, and a bit of secretory component there. And that is also detected by the anti-secretory component antiserum. But of course, none of these other antibody forms are found either. Now, the assembly for secretory antibodies is actually rather efficient, and the accumulation is surprisingly high. We estimate something like 5 to 8 percent of total soluble protein in transgenic tobacco leaves. We originally believed that the antibody was being treated in exactly the same way as the IgGs that we already expressed in plants and that these things would be secreted into the stable environment of the AMOPLASTIC? space.

That actually has not been the case now, and we have more recent data that suggests that this molecule gets stuck in the endoplastic reticulum. A small amount is secreted, and a small amount is diverted to the vacuole.

Now, we're not entirely sure why that is the case. In fact, it's not a function of the assembly requirements in the secretory IgA because this phenomenon was also seen with diamer as well as the monomer. An obvious solution would be that is a sequence in this construct, a heavy chain presumably, that targets or retains the antibody in the ER or even targets it for vacuole liquidation. But although not many sequences of this type are known, we haven't been able to find any of the ones that have been published. We feel this may be more of an assembly quality-control kind of problem, but the bottom line is that the accumulation is still five to eight times greater than the secreted form of IgG, and so we feel that the accumulation within the endoplastic reticulum might not be as disadvantageous as we originally thought.

Functionally, this antibody appears to do everything we hoped it would do. This is a dimer? Dimer analysis of antigen binding. On this kind of assay, you don't see the added benefit of the higher avidity of secretory body, but the functional sort of the affinity for adjuvant binding is equivalent between the part secreted IgA and the IgG, and the only difference that you see here between the two lines is down to the size difference between the two molecules.

We can do competition ELISAs as to show the functional avidity difference between these two antibodies, and these are as predicted One of the potential advantages of the secretory antibody was that it should have a longer half-life in the mouth because it's less prone to proteolytic degradation, and we have shown this to some extent in human volunteers where

we just apply a single dose of antibody. Here's the RESINENCE? time of IgG. You can see that after -- that can be sharper a little bit, please. You can see that after single application, really, you cannot detect any antibody after that 24 hours whereas for the serum IgS or secretory IgA, there is a rather point here. The last time we see functional antibody is at 72 hours, which is three times the RESENCE? response of the IgG.

We have carried out a recolonization prevention study in human volunteers, and this was sponsored by part of our technology. This study is a little complicated. What we do is we select people who already harvest Strep mutans in their mouths and therefore would be susceptible to dental caries.

Our previous experience show the monoclonal activated antibodies are not sufficient to Strep S mutans which is already colonizing the oral cavity. Sorry. Can you go back and just focus that, please? And given the way it works in possibly interfering with adherence of the bacteria, that's what you might expect. So we have to go through initial process of antisepsis using a commercially available preparation of Chlorhexidine. After that we applied the monoclonal antibody to Patient C, and you'll see here that we have regime which is two applications a week for three weeks, and then we monitor the recolonization of Strep mutans.

This is the data from our study. We've looked in dental plaque, but I'm just going to show you data from saliva, which is actually a better mirror of what's happening in the mouth.

You'll see that the initial antisepsis regime does bring the Strep mutans down from what we arbitrarily call 100 percent down to undetectible levels.

Why, I hear you cry, don't we just treat patients with Chlorhexidine all the time? Well, the reason is it causes mouth ulcers and other irritation, and people don't tolerate it unless they live in Sweden and there's nothing else to do. The Swedish appear to be the only group who use Chlorhexidine on a regular basis. If you just stop the Chlorhexidine at this point then, you'll find that Strep mutans does come back rather particular quickly and reaches the original levels after about two to three months. This happened in both of our control groups: saline group and the group which we gave plant extract spiked with nonspecific bovine IgG.

In our experimental groups we've compared the secretory antibody extracted from plants with the Guy's 13 IgG which was derived from mice ascites, just like the original experiments we did all those years ago. And what we see here is that during the immunization phase, there is no recolonization by Strep mutans. And in common with our previous studies, we show that this is a long-lasting protection which in this study went out to 120 days but in previous studies have gone on for one to two years.

Now, this is completely at odds with what is generally believed to happen in passive immunization, say most of you know, I'm sure, that passive immunization is generally thought only to give protection once antibodies are around. And as I've already shown you, antibodies

don't hang around for much more than three days. What we believe is happening to prevent -- just before I go on, I should say we looked at the control organisms. Now, it's difficult to go through all of the other oral bacteria in the mouth. We've picked up ANTIGEN GLUTEI? in previous studies which have looked at Streptococcus SANGWIS? and found other species, and we show that the antibody specific for Strep mutans has no activity against these other organisms.

So what we think is happening is that we are reducing rather specific alterations in the oral flora. If this is the mix oral flora on the tooth at the start of the experiment with Strep mutans here in red, the antisepsis regime, of course, is nonspecific in all of the bacteria, which then all try to recolonize as soon as you start the antisepsis. And by applying antibodies directly against Strep mutans, we are specifically inhibiting recolonization only of that bacterial species. And we're giving over three weeks of them -- sorry. Can you go back? Turn it on perhaps. So what I'm saying is that we're giving a three-week jump on the other oral bacteria to recolonize. And then, of course, one or perhaps several of the bacteria will shuffle up and occupy the niche which was originally occupied by Strep mutans. And so you can stop the antibody application, and you have a natural bacterial colonization resistance against further Strep mutans colonization.

Now, we don't have any evidence for this. It's going to be a hard thing to prove. The only thing I can say is that in the few volunteers that we did manage to follow more than two years, we found that recolonization did occur in those people who took a course of antibiotics in the interim.

One of the problems with using dental students is they all tend to disappear after two years and go and earn some money, so in the future one of the qualification criteria for getting into our study, I think, will be a poor academic record. This has finally convinced me to go to computers now. I was so impressed by everyone's presentations yesterday. Well, what I was going to tell you then is that of course, we have looked rather hard for adverse effects from this approach. Now, one might not expect to see many adverse effects because we're not delivering this compound systemically, but on the other hand, the oral cavity and the oral mucus membranes are rather sensitive tissues which you might expect would react adversely if there was anything in our product with problems. Thank you very much. It doesn't matter.

The first thing that we've looked for is clinical irritation, any evidence of inflammation, PAXIDEXIS?, gingival indices, and so on, and we really did not see anything of this kind. I should tell you that the antibody was reasonably well purified by chromatography before we went in.

We've also looked for standard immunological indices here and see nothing. These are the clinical ones. And we've also looked for a number of immunological indices to see whether application of this plant-derived recombinant protein would induce either serum, salivary mucosal, or local gingival fluid antibody response, and again, we've seen no reactions of this

kind. The point is, of course, that we put foreign immunoglobulins in our mouths all the time whenever we eat or drink, and of course, we put compounds in our mouth all the time. But one of the big issues that has always popped up is the glycosylation of plant-derived proteins, and really, to try and resolve these issues, we have done a laboratory study with LOIS FRY? at the University of RUAN? where we have compared the structure of the glycans in the mouse-derived version compared with the plant-derived version.

Now, I'm going to go back to IgG here because of course, the glycosylation is much simpler. The mouse immunoglobulin has rather standard glycan structures depicted by these four complex glycans and really nothing surprising here. The only thing that is different about this antibody which is in common with, I think, 30 percent of Immunoglobulin G is that there are two glycosylation sites, the standard one at the hinge ~~HINGE~~ region and a second one at the antibody site. Most of those glycosylation sites are utilized both in the mouths and in the plants. Now, the glycan structures that we saw -- sorry. This is rather dim, but I don't really want you to see the details. I just want you to see the fact that it's rather more glycan structures in the plant.

These are the plant glycan structures, and the first thing that you notice is there are actually rather greater heterogeneity of glycans, and in fact, we see a lot of structures which are intermediates between the man five to man eight, so these are not completely processed glycans.

Now, one of the reasons I felt that this was happening is the way we extract the antibody. We grind up the plant tissue, and so I felt that we may be taking out some antibodies which were in transit within the endomembrane system. The complex glycans shown below here are very simple, very small, and in keeping with previously published data for plant glycoproteins so you get these GOLLID? residue here, GOLLID? 1-2 and the Alpha 1 through residue here and rather small additions of the ends here, not the Lewis A antigens that have been reported in other plant glycoprotein. Now, we asked then the question was, well, yes, of course, we expected these glycans to be different in plants, but are they going to be immunogenic in humans or in mammals? The dogma has always been that plant glycans are rather immunogenic. I'm not sure that that's necessarily true.

As you may know, PLANT HYDRIC? structures in themselves are poorly immunogenic unless they're repetitive sequences. And the second thing is, of course, in these glycoproteins you are presenting plant glycans but on a mammalian protein.

So to address this issue, we've gone back into experimental mice and immunized with the plant recombinant protein. And what we've looked for then is the ability to raise any kind of immune response against the recombinant glycoprotein. Can you focus that a little bit for me? Thank you very much. As a control, we've used oxidized perioxidate in standard plant glycoprotein, and immunization was done on three occasions. And to try to optimize this, we've also given alum as an adjuvant. And you can see here on the glyco analysis that we get rather

nice antibody response against HRP, but here you'll see we have no response against the plant recombinant IgG.

We've done some ELISAs too, althoug those are difficult to take to the mouse IgG one response when your adjuvant is also an IgG one, so all we can show is really the IgG 2A response and an anti-light chain response, and that's why we use the sort of primary testing base.

Again, against HRP, you get very good antibody responses. In our group immunized with the plant recombinant IgG, we saw very little response. In fact, only two out of six animals did we take any antibody response. It turns out that this response is not targeted towards the recombinant IgG or its associated glycans because we couldn't compete it away with any relative antigen, and I think these responses are more towards contaminating plant proteins which got through our GLUFICATION? procedure. So the bottom line on this then is that my feeling is that when presented as a self-protein, these plant glycans are really not as immunogenic as people have previously thought. Of course, these studies will have to be repeated in humans because the only other system where people really looked is in rabbits, and that's where all of the anti-PAR? Glycan antisera are available have been raised, and as you may know, rabbits are rather promiscuous in their abilities to produce antiserum.

There are other things I don't have time to tell you about, but these are really sort of shown in this summary slide. We have made a number of antibodies. I've only really talked about secretory antibody in plants today.

We know that these antibodies need to go the endomembrane system. This is a very important aspect of expressing full-length antibodies in plant. And the reason they have to go through that system is because in order to fold and assemble these things, they have to interact with the ERS chaperons of which BIP? and PDI? are two of the more important ones. And we've also shown the importance of this interaction. The assembly of IgG differs from the a assembly of the secretory IgA as a LIPID?. Both of those are glycosylated, and both can be extracted very readily from plants at reasonably high levels, although we do feel that there is room for improvement in both cases. Functionally, we've not been able to show any difference between the mouse-derived antibodies and the plant-derived antibodies except in this structure of the associated glycans, although as I've said, we don't think these differences are going to be that significant.

We've done the clinical trial using the secretory antibody. In fact, we are now completing a Phase 2 trial in UCSF?, again, with collaboration of plant technology, and our results to date indicate there are no adverse side effects.

I finally would like to take 30 seconds to tell you about other applications which we see as a result of our experience using antibodies in plant, and one I really want to talk about is immune complexes because one of the things I've learned here is although lots of people are

expressing very good antigens in plants for vaccines, nobody has really addressed the issue of adjuvanticity and how to produce the immune response, particularly for oral delivery. Now, we started this project because we were interested in two aspects. One is that we notice that many antigens are poorly expressed in plants, and yet the first time we tried to make an antibody and many other people, it's true antibodies expressed enormous levels. And we believe this is related to the induction of chaperons and their association in the ER.

So we've started making a number of constructs where we've fused an antigen with parts of an immunoglobulin heavy chain. Now, the adjuvant we're using is GP 120, which is notoriously difficult to express in transgenic plants, and this ELISA data shows you our accumulation levels, which are really not even above base line. We can barely detect it.

When you start to do other things like sequence on GP 120, you don't get much improvement. And then we started adding a CH-2 domain on the interim GP 120, and well, if you close your eyes, you might imagine there's a slight improvement. But when you put two immunoglobulin domains on, then we start to get a bit more excited. And then when you put the entire heavy chain on, the interims of GP 120 and then cross that plant with a light chain plant expressing -- a light chain plant for this IgG, but then we've got expression levels of GP 120 which is equivalent to 1 percent expression levels of the IgG.

Now, the first advantage of that is that we see this as a way of boosting expression of antigens which are otherwise difficult to express in plants. We don't know why this is, but you know, one idea is that maybe taken through the whole endomembrane system much faster.

If you just want GP 120, of course, then you can cleave it provided you put it in the right cleavage site. But we feel a big advantage here is that if you make the immunoglobulin, more of this molecule specific for your antigen, then you're also making an immune complex. And traditionally, new complexes have been known to be highly immunogenic for vaccination. And not only do you produce this kind of immune complex, but there is also the capacity in plants to form multiple complexes. And using this construct in GP 120 and anti-GP 120 monoclonal antibody, we're going to be going into mice in the next two months to look at comparative immunogenicity with GP 120 alone. So more of that later. Finally, I'd like to introduce you to my colleagues, a small but hard-working group: my two post-docs, Pascal Drake and Daniel Chargelegue who are responsible for the transgenic plants and protein certification and immunization studies and my two students, Nicholas Vine and Craig van Dolleweerd who have done all of the antibody engineering work.

Thank you very much for your attention.

KATHRYN STEIN: Thank you. I think we'll steal five minutes from the coffee break and have ten minutes for questions. Would the other speakers, Dr. Jilka and Dr. Mason, come up and if we could have the lights up. I'd like to ask a question while people are going to the microphones and the other speakers are coming up. Normally IgA has a higher percent by

weight carbohydrate than IgG. What did you find on the total weight of carbohydrate in your IgA antibody compared to your IgG?

JULIAN MA: We haven't looked at that yet. As you may know, actually, our IGS antibody is a true IgA chimeric molecule consisting of two IgG domains fused to CF2/CR3 to maize, but in our newer constructs we were just making IgA without looking at them. Yes.

KENT CROON: My question is to Dr. Julian Ma. It's about monoclonal antibody technology in plants and how you're going to be addressing the question of antigen variation, the variance of pathogens that might not be found by the antibodies that you express in plants.

JULIAN MA: Well, that's not a question, of course, that's specific to plant technology. I think it's specific -- it's one that addresses anybody to making antibodies, and I'm the first person to say that plant-expressed antibodies are not systems to be screening for effectively antibodies in. You have to choose the right antibody before you go in. With relation to the caries antibodies, what we've done here is to target a very important epitope. And our evidence is that if there's any mutation in this epitope, then certain mutans will lose its virulence in any case. So selection of antibodies before you go into plants is one of the most important aspects.

EZIO BONVINI: This is Ezio Bonvini from Center for Biologics, a question for Dr. Ma. Have you looked and seen whether there is any induction of ~~A TYPIC~~ atypic antibody with your oral IgA?

JULIEN MA: We've looked for antibodies in our IgG studies, and we didn't find anything.

EZIO BONVINI: Thank you.

GORDON MOORE: Two related questions.

KATHRYN STEIN: Could you state your name?

GORDON MOORE: I'm Gordon Moore from Centocor. You indicated there was a protective response to your antibody, but the data that you showed was recolonization data rather than caries data. So the first question is, do you have any evidence that there's an actual reduction in the absence of caries in your treated patients? That's Question No. 1. Related question, how strong is the evidence that Strep mutans is really the only bacteria that's involved in induction of caries, and do you worry that the blocks to mutans, other bacteria will come?

JULIAN MA: Good questions. The answer about the protection of disease in humans is a difficult one to look at because it takes so long to develop caries, and in fact, the Phase 3 trial that we are planning is planned for at least four to five years.

Even worse, in the dental student population, despite the amount of chocolate they seem to eat at lunchtime, doesn't get much caries.

All of our protection data with this monoclonal antibody against Strep mutans is derived from our monkey studies, and studies in rodents, too, have shown that we do get a reduction -- a significant reduction in protection against dental caries. Those studies will have to be done in humans. In relation to other organisms, that may cause dental caries, well, yes. If you look through the literature, you will find probably three or four major candidates as cariogenic organisms. Strep mutans stands out like a beacon amongst those, and although we could argue until the cows come home about the other organisms, really, you'll only know that until you have -- until you have a way of only eliminating Strep mutans that of course we've never been able to do until now. My feeling about the other organisms is yes, they may contribute partly to dental caries. Many of them are secondary colonizers, so they are attracted to the acidic environment that Strep mutans generate so -- and then, of course, dental caries is not just one disease. There are many variants of dental caries, some of which may in -- some of those other organisms may be more important.

But my feeling is if you eliminate Strep mutans, then you will reduce dental caries by up to 90, 95 percent. You won't eliminate it all together.

KATHRYN STEIN: Yes.

MICHAEL HANSEN: Michael Hansen, Consumers Union. This is a question for Dr. Mason. I'm curious to know what kind of safety testing has been done on the potatoes or that will be done on the tomatoes, not looking for acute effects but long-term effects that might be a result of the engineering process itself. Do you have any kind of rodent studies or anything else where you're looking for some of these other effects, or are you only basically concentrating on whether you see this antigenic response?

HUGH MASON: Well, today we've only focused on the latter. Obviously, there needs to be further safety testing done. I'm not sure exactly what type of testing you might be suggesting.

I think that's pretty well established that there's not going to be much danger of the DNA, the recombinant DNA. I think we have to be concerned about effects on allergenicity or perhaps development of oral tolerance certainly.

So certainly we do have plans to study the development of IgE, although so far we've not attempted to measure that. But certainly, it's an important thing that we need to focus on in the future. And of course, our studies on optimization of dosage regimen and frequency, we will address the oral tolerance issue.

KATHRYN STEIN: I have a question for Dr. Mason and Dr. Jilka as well. One of the things that concerned me a little bit about the data we saw yesterday was the regimens that were used to immunize with these products and in particular the close proximity of the immunizations, Days 0, 7, and 14.

One can understand that for an infant diarrheal disease you have to deal within that time frame, and you have to immunize to protect against the disease, which only occurs in the newborn period. And that will be a question for human infants as well.

But for vaccines designed to protect against diseases that don't occur during the newborn period, I think it would be of interest to explore regimens that involve immunizations, say, similar to ones used for parenteral vaccines with a longer spacing to see if you get better boosting. Clearly, some of the challenge experiments showed that a delayed challenge, you got a good booster response. So I'll actually be interested to hear from both of you on whether you've explored any regimens that involve immunization with a longer interval.

HUGH MASON: That's a very good question and we have not addressed that. Our clinical studies have been relatively small scale, and certainly we'd be interested in doing a longer phase study like that to examine how long after the initial priming and/or boosting we can hope to get protection. So far we haven't done any protective studies or challenge studies.

As I said, we're hoping to do some studies later this year that may be able to address challenge with Norwalk virus. Hopefully we can include very long term, perhaps one year after immunization, to evaluate whether there's any protection or anamnestic responses during that time scale so --

KATHRYN STEIN: Go back to some of your previous volunteers, I guess, and boost them.

HUGH MASON: That's a good point. I'm not sure if we'll be able to do that, but that's certainly a good idea.

KATHRYN STEIN: They've all graduated from medical school.

HUGH MASON: Yeah, exactly.

KATHRYN STEIN: Dr. Jilka, would you like to comment on that?

JOE JILKA: I would pretty much second what Hugh said. Our 10-day regime that we gave them was pretty much a wild guess as to what, but with the next set of trials, we're going to look at different timing.

And certainly in the case of animals, one really has to look into the conventional husbandry techniques to see where it can fit in best. You know, especially food animals, you know, they don't live that long, so, you know, it just has to fit with that, and we have to do those studies yet.

KATHRYN STEIN: Last question.

PATRICIA SHEWEN: Thanks. Just a quick question for Dr. Ma. In the dairy end when you talked to the antigens made with antibody, what's the isotope of immunoglobulin?

JULIEN MA: We have -- the one I showed you is a human IgG one, but we also have two other model systems where we're using immune year IgG 2-2 and UNIMERE? IgG 2-2 with other different antigens.

PATRICIA SHEWEN: Enhanced immunogenicity. Do you know if that's indicated, or is it particular antigen?

JULIEN MA: It could well be FC mediated. It could also be related to epitope protection during processing in antigen-presenting cells, and that's one of the reasons we've also made these constructs with just the CH2/CH3 domains in them.

PATRICIA SHEWEN: Thank you.

KATHRYN STEIN: I'd like to thank all the speakers this morning, and we will reconvene at 10 o'clock.

(Short recess.)

DAVID ESPESETH: I am retired, but prior to retirement I was Director of the Center for Veterinary Biologics in the U.S. Department of Agriculture, responsible for licensing and policy development for veterinary vaccines. And I have to say that prior to this seminar, we didn't know much or I didn't know much about what the status of the science was on production of biological products in plants. We've had some excellent papers in this morning's session, and it really impresses me as to the amount of good science that has gone into getting us this far. But as I think back when we were developing and involved in the licensing of some of the first recombinant-derived vaccines, we were very excited in 1980 and '82 when these products were beginning to be presented for review. We were thinking at that time that in the next three to four years we would really be seeing these products on the market.

In reality, we are just now seeing a large number of recombinant-derived vaccines coming to the market, and it wasn't until 10 or 15 years after the first ones were presented that a large number of them actually were commercialized and became licensed products.

So I guess what I'm saying is, even though the information that we've heard to date demonstrates a lot of promise and stimulates a lot of excitement for the potential of these new methods that we have to produce biological products, there is still a period of time needed for these products to be evaluated and developed before they will reach commercialization. One of the key factors in that road to commercialization that will determine which products make it to market and which ones don't is the regulatory requirements that will be established for this group of products. I think, although government regulatory officials tend to be modest, they are really going to play one of the most important parts in how these products will be developed and which ones will be developed by the standards that they set for them. I think this session on regulatory considerations related to plant-derived biologics is probably one of the most important and should be the most interesting to industry as we begin to talk about establishing a clear regulatory pathway for these products so that those that are interested in commercialization can prepare and plan properly to bring these products to the market in an efficient manner. We're very lucky today to have three of the regulatory officials from three of the government agencies that will be responsible for establishing the regulatory framework for these products with us today to give us their current thinking. It's very encouraging to me that the approach that is being taken to develop these standards is a cooperative effort between USDA, FDA and PPQ along with the industry. I think we're off to a good start as reflected in this meeting, and hopefully, we'll hear some good information as to what the future holds.

Our first speaker today is Dr. Jim White. He's with the U.S. Department of Agriculture, APHIS Plant Protection and Quarantine Biotechnology and Biological Analysis Unit.

He has a B.S. in biology, an M.S. in microbiology from Florida Atlantic University, and he received his Ph.D. in botany and plant pathology from Michigan State University in 1976.

He supervises a team of scientists in PPQ that reviews applications for field-testing and commercialization of genetically engineered transgenic plants and microorganisms and is also responsible for developing the regulatory policy that permits the release of these products for commercial production. So Dr. White.

JIM WHITE: Well, you can see we have low bid in the government, and I'm using old technology, so we're going to raise taxes. We're moving to PowerPoint. I want to remind everybody that please besides picking up the evaluation forms which has our names on here -- and this is the one thing that we didn't discuss. I guess isn't going to my supervisor, how my performance is today. But there's one thing that you need to look at if you're going to be at the round-table discussion at three today.

Out at the registration desk, there's this one-page thing developed by the working group on how the three agencies envision coordinating regulations of these products. And so you should look at this over lunchtime. If you have any questions, that can come out at the round table this afternoon. As Dave said, I work at Plant Protection and Quarantine group. We regulate transgenic-engineered plants under the Plant Pest Act of 1957 and the Plant Quarantine

Act of 1912. Louise Henderson will talk about the Virus-Serum-Toxin Act that they regulate these products under. So what are plant pests? Well, first of all, they're any living stage of insects, mites, plant pathogens, a variety of organisms, plant viruses, which can directly or indirectly injure plants. So we have a very broad definition of plant pests.

So within APHIS and PPQ, we've got all these in PPQ. Start writing regulations on biotechnology. In 1987, just shortly before I joined biotechnology group, we published our first regulation on introduction of genetically engineered organisms. Mainly, that dealt with field-testing of transgenic plants and microorganisms.

In 1993 there was another regulation, called for notification procedures. This was a streamlined paperwork reduction for field-testing. And the second part was petition for nonregulations status. That sets the regulations for the commercial release of engineered plants, for example, like RoundUp Ready corn or the Bt corn. In '97 we had another modification for notification for field-testing of engineered plants. All these regulations are available at our Website. So what is the definition of what we regulate? So the legal definition is for any regulated article, it's any organism that's been genetically engineered from any organism that's a plant pest. And so that includes definitely a recipient like tobacco mosaic viruses in plant pests, or if you use Agrobacterium, Agrobacterium is a plant pest that's in the vector or vector agent or any sequences derived from plant pests.

But Part B of the definition is any product produced in engineering which the Administrator determines is a plant pest or has reasonto believe is a plant pest. And our policy is that all plant-derived biologics fall under either one of these and would be regulated by APHIS.

And you might wonder why. Because we believe these are -- they have indirect injury to plants because of course, one major issue would be their inadvertent mixing with products, say, for example, corn that was intended for animal or human consumption. So these kind of field-tests would be regulated by APHIS. So what experiences have we had with field-testing? Here's our little graph. This is to the end of February. I started back here in 1987 where we issued by permits. Last year we had nearly a thousand authorizations for field releases.

As I said, in 1993 we instituted this system called notification and was modified in '97 to include all plants, virtually all plants. And so virtually 99 percent of the all field-testing of engineered plants go under the notification system.

So what doesn't go under notification systems are genetically engineered microorganisms and especially in plants that are producing biologics. One of the criteria which I'll show for notifications -- and there are a variety of them. I'm only showing the one that's appropriate here -- is that under the notification system, this streamlined system, one criteria is the introduced material does not encode products intended for pharmaceutical use. In essence, the working group has worked together. We have a clarification on what that means because

the same products could be intended for different uses. For encoded products intended for pharmaceutical use, if their commercial use would require approval from the Food Drug Administration, from CBER or CDER or any other FDA group except CFSAN or would have to have approval from Center for Veterinary Biologics here, then your plants have to go under permit.

Well, we've had plant-derived biologics. Our first field-test was BioSource in 1991. And they're now Large Scale Biology. Larry Grill said that we won the award for the most controversial field-test we had in the 1990s.

In 1992 the Noble Foundation in Oklahoma had the first field-test of a plant for biologics, and that was alfalfa. Here are the companies. I think you all know who those are now that had done field-testing of plant-derived biologics in 1991. The goal of all field-testing for transgenic organisms in APHIS is to ensure that the field-test is contained. And here's what the goal of containment is, is to have a controlled release into the environment under conditions so that no transgenic material will persist in the environment and that any unintentional or unexpected effects, if any, can be confined to the test site and managed in such a way that there is no environmental risks after the field trial is terminated. That's a goal for all engineered organisms for your field-tests.

So I'm going to talk a little bit about containment procedures that we've developed, and we have experienced one for corn and the other for tobacco mosaic virus.

So how are the containment protocols developed? Well, the applicants submit containment protocols that APHIS and the states review and modify when necessary to ensure a contained trial.

I will say for every authorization for the interstate movement, the importation in a field-testing of all transgenic organisms, those applications go to the appropriate receiving states, and they must concur before the action authorized. These decisions are made on a case-by-case basis for these permits for plant-derived biologics. Now, how does APHIS ensure that containment protocols are being followed? APHIS inspects all field-tests conducted under permits. Last year there were 24 of them. Inspectors went to all those sites at least once. In fact, I went to the BioSource site to do that inspection last year. And the states may also inspect these field sites.

Let's talk a little bit about containment protocols for maize. Of over the 5,000 field-tests that we've had, maize is the most common field-test organism. John Hammond talked a little bit about the biology of maize, and we have no relatives in the United States.

So the containment protocols that have been used for maize for more than a thousand field-tests, the plants can be harvested before they're flowered. The reproductive structure can be bagged. That means actually putting paper bags over the reproductive structures. There's

temporal isolation, and as they talked about that yesterday, that was briefly mentioned where you plant your corn at a time where other corn is not flowering. One of the conditions for temporal isolation is that people have to walk the fields to ensure that the nearby corn has completed its shedding pollen, and if not, they have to remove the reproductive structures by hand. The last way is the doubling of the American Association of Seed Certifying Agencies isolation distances from 200 meters to 400 meters. That's from 660 feet to 1,320 feet.

Well, what are you going to do with the remnants? So if you have your harvested corn seed or your product, we have to get rid of the stalks. Well, here are three common disposal options that have been approved.

All living material can be sprayed with a herbicide -- for example, glyphosate allowed to die, and then allowed then to be plowed in the field for decomposition.

All living material can be disked into the soil directly without being sprayed. The plant material can be harvested and taken to a pit in the field for burial and covered with at least 6 inches of soil. It's not vegetatively propagated, so this is why these options can be done. Certain crops that are vegetatively propagated, we would have different disposal options. Now, after you dispose, you still have to monitor those areas where there are materials. And that's only if you produce -- if seeds were produced, then the field needs to be monitored for volunteers.

Field-tests are often done for two seasons in Florida, Puerto Rico, or Hawaii. Where temperatures for growth following the harvest are warm enough to germinate corn seeds, they can irrigate or do such a manner to stimulate germination. And then any seedlings that are found can be destroyed either by hand, disking the soil, treating with Round-Up or some other herbicide.

In cooler climates there was a field-test here in Iowa last year. The field should be monitored for volunteers this spring. And again, any seedlings that emerge have to be treated like they would be treated up here. We've had several firsts with viruses. The first engineered animal virus vaccine, Pseudorabies, was approved by Center for Veterinary Biologics in 1988. In '86 the first recombinant human vaccine was approved by FDA. So as I said, the first field-test for engineered virus took place in 1991. I'm going to talk about that. You had a series of other virus field-tests in the '90s. I've left out that there was a series done by R. J. Reynolds who was collaborating with BioSource.

We've had field-tests for TMV for most of the '90s. They did a single test for tobacco etch virus in 1998. But compared to plants, virus containment issues are much more virus specific and site specific and plant specific than for corn. And I'm going to spend some time because there was some discussion yesterday about containment for viruses.

Well, first of all, generally the plants aren't transgenic, and they generally don't flower. Usually TMV-resistant tobacco plants have surrounded the outer perimeter of the experimental plants. They act as a trap. The important part for site-specific test is the potential for the presence of Solanaceous weeds that are hosts for TMV. These are often, like, horsenettle, black nightshade, and ground cherry are controlled on the site by either herbicide application and rogueing. As I said yesterday, that's why I went there, to ensure that there weren't too many weeds present. A little bit about the biology of TMV. Again, it overwinters mainly in tobacco plant debris and possibly Solanaceous weeds. That's why we're concerned about weeds. It's mechanically transmitted. There are no insect vectors known.

And as I said yesterday, in common its natural host range is significantly smaller than the experimental host range, it's been reported in the literature.

So what can we say about the differences between engineered viruses and the nonmodified virus? Well, the engineered viruses are less stable, as the virologist said yesterday. It slowly reverts to essentially the nonmodified virus. BioSource submitted data that show that the host range is not altered by the engineering. The symptoms on the plants are different than the nonmodified virus while – and they're different from different constructs which does aid in identifying or noticing that there' something unusual in the field-tests And when you put the nonmodified virus with the engineered virus in the midst of infections, by directly doing that, the nonmodified wild-type virus outcompetes the engineered one, which says the engineered virus is less fit. It has competitive disadvantage.

So moving on to monitoring and data. Data reports are due from BioSource prior to the issuance of the next permit. They submitted data to APHIS that show that the decontamination procedures for removing the virus from the farm tools are adequate to control the virus.

As you saw in BioSource's presentation yesterday, they have those harvesters and trucks that move this stuff. They had to ensure that there's no virus present.

The modified virus movement is limited from plant to plant to the field. Occasionally they have found, you know, the removal of Solanaceous weeds is not 100 percent, and they've left some of those there to show that virus can move in the field, but they haven't seen them move outside the field. That's probably because people do walk the fields, and virus is mechanically transmitted. Trap plants and weeds adjacent to the field are assayed for virus, and none have been found I'd like to then conclude my talk to talk about how APHIS, Center for Veterinary Biologics, and FDA are going to coordinate this besides working on this meeting. We recently implemented procedures so PPQ will provide CVB and FDA confidential copies of all permits for plant-derived biologics. That includes importations, interstate movements, and all field-tests. PPQ will continue to perform site inspections and provide the appropriate agencies with the inspectors' reports for those.

And so in conclusion, I think we have experience with over 5,000 field-tests of engineered organisms in the field with no significant unanticipated or unexpected events based on our inspections and reports from our companies, so we feel that we've had experience that field-testing plant-derived biolo proceed safely as long as there's continual vigilant oversight of those tests. Thanks.

DAVID ESPESETH: We have a few minutes if you have some questions for Dr. White.

JIM WHITE: Fill out your form.

DAVID ESPESETH: Fill out your form. You gave us a good idea of what would be the mitigating procedures used for field studies with tobacco mosaic virus and some things, but how about the process? What kind of a process do you go through in terms of risk assessment?

Is that done on every application, or is it done on each type of product that comes along, and what can they expect to face in that area?

JIM WHITE: Now, are you talking about environmental assessments for each of those kind of stuff?

DAVID ESPESETH: Yes.

JIM WHITE: We've done environmental assessments. Things have changed on our -- we've been implementing policies, and we have done environmental assessments for the BioSource field-test several years ago when it moved to Kentucky. We've been tiering to that environmental assessment. Contained field-tests are exempt from NEPA considerations. I guess the other agencies can talk about when their NEPA implementing regs will be triggered for that. I would just maybe -- should say one thing. We never -- I wouldn't say never because we don't know all the potential products, but currently we can't envision that these products would be deregulated through the petition process like Bt corn. They always are going to have oversight from APHIS, so we're not going to go through that process.

ROBERT MILLER: I'm Robert Miller, private citizen.

JIM WHITE: So who gets to say that he used to work for APHIS?

ROBERT MILLER: You very subtly slipped in permits for interstate movement and international movements. Does your agency have any authority over these plants that do not move interstate?

JIM WHITE: Within their state? Okay. You know, our regulatory authority goes to interstate commerce. We don't have authority for intrastate movement except all research

should be under the NIH guidelines. The other regulatory agencies can talk about that. Our group has talked about that. You have to work under the NIH guidelines. I have told people when they've asked about those issues that it's best for them to tell their agricultural official whose names and addresses and phone numbers are on our Website to let them know that they're shipping them, for example, say, from their facility in Beltsville to the NIH campus in Maryland.

We do actually see quite a bit of things because when we get applications, if you're shipping Agrobacterium containing TGEV genes which you need a permit for, we ask always what are you going to be doing with those Agrobacterium constraints? So a lot of that stuff has come indirectly as that.

But to my knowledge I think everybody is following the NIH guidelines. From Dr. Koprowski at Thomas Jefferson when McGarvey was there, they were going to build a greenhouse, you know, have authority over -- that was sort of an intrastate thing. They did ask for advice. We talked with Pennsylvania, the state, on the design of a contained facility.

DAVID ESPESETH: Any other questions on environmental concerns or other issues that PPQ would deal with? If not, thank you very much.

JIM WHITE: Thanks, Dave.

DAVID ESPESETH: Our next speaker is going to talk to us about regulatory requirements for veterinary vaccines. Dr. Louise Henderson is with the U.S. Department of Agriculture, Animal Plant Health Inspection Service, Veterinary Services, Center for Veterinary Biologics, Licensing and Policy Development.

She is chief staff microbiologist of the biotechnology and diagnostic product section in licensing and policy development. She has a B.S. and an M.S. from Iowa State University and received her Ph.D. from Iowa State University in molecular, cellular, and developmental biology in 1993.

In her current position as chief staff microbiologist, she is responsible for regulatory policy development for both diagnostic products and products derived through biotechnology.

And so Dr. Henderson.

LOUISE HENDERSON: Thank you. Well, unlike Jim White, I'm not going to be able to tell you a lot about our experience with these plant-based vaccines because we have no licensed plant-based biologics at this time. However, we're very well aware that we are likely to get applications soon, and we are working, as you've heard, with FDA and PPQ to establish a common framework for our regulations.

And as Dr. White mentioned, we are all very much constrained by the authorities under which we regulate. We are constrained by the Virus-Serum-Toxin Act, and all of our regulations are based on that Act.

Part of that Act gives us the right to regulate biologic products, and our current definition of a biological product for the Center for Veterinary Biologics. The definition states veterinary biologics are all viruses, serums, toxins, or analogous products at any stage of production, shipment, distribution, or sale which are intended for use in the treatment of animals and which act primarily through the direct stimulation, supplementation, enhancement, or modulation of the immune system or immune response. Now, veterinary pharmaceuticals are regulated by a branch of the FDA Center for Veterinary Medicine, and they will regulate all products that are used in the diagnosis, cure, mitigation, and treatment or prevention of disease like antibiotics and NSAIDs, articles other than food intended to affect the structure or function of the body of animals. They will regulate products that are intended for contraception, anesthetics, drugs, and hormones.

The Center for Biologics -- the CBER for the FDA biologics definition is very similar to ours: Any virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component, or derivative, allergenic product, or analogous product applicable to the prevention, treatment, or cure of disease or condition of human beings.

So as you can see, both of our products are very similar except that CVB does not have authority to regulate blood and blood products. We believe that plant-based diagnostics and vaccines should be held to the same standards as those for other products. But we have to adapt those requirements to the technology. So we may need additional regulations for these types of products, and we will be writing those regulations as we can develop what we believe are reasonable requirements. One of the reasons I can't tell you a lot about what those will be is that the intent of this meeting was for us to have this dialogue prior to establishing our regulations so that we would have adequate input before we set down our regulations in the 9 CFR.

Licensure requirements prohibit the sale of biologics that are worthless, contaminated, dangerous, or harmful. Thereby, we always insist that products are pure, safe, potent, and efficacious.

Both the FDA and USDA use these standards to determine whether product is ready for licensure. And all of the new technologies must meet the intent of the regulations that are for conventional products How will plant-based vaccines be regulated? Well, it's a good question. We're working very hard to determine exactly how best to regulate these products. The standards are under development, and we certainly will be working very closely with the other two groups, PPQ and FDA, to ensure that we have a good basis for all of our regulations. Also, within the Center for Veterinary Biologics, we have a coordinated review team that is doing its best to determine the appropriate way to apply regulations with very specific details as

to how we will deal with each of the types of questions we have for regulation of veterinary biologics.

Regulation will include prelicense data evaluation and testing, oversight of production processes, and post-licensure monitoring, much as we have for our current products.

And we do this through a number of different methods, including inspection in vital product testing and approval, both prelicensure and post-licensure, of any changes you would like to make to your production.

We will keep control of biologic products, much as we do for our traditional products, thereby alleviating some of the concern about deregulation of these products. Not only will PPQ not deregulate these, but any product that is a licensed product will have to maintain a record of its production and the ultimate fate of all of its produced vaccines. We'll try to develop policies that will help us deal with all the different types of plant-based biologics we expect to see. Certainly the feed-based vaccines for oral immunization are ones that we are talking mostly about because those are the ones that we're seeing mostly in the literature now.

But also, we realize that purified protein production is an important part of plant-based technologies, and we may see these for both parenteral subunit immunization or for feed additives.

Also, plantibodies, we expect to see applications for products using antibodies produced in plants and maybe diagnostic kit

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components that are produced in plants, either the

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plantibodies or the antigens. Primarily, I'll focus on talking about the oral products since those are the types of applications that will require some different regulations. These other types of applications very well may fit quite well into our existing policies. We have a master seed and a master cell concept, and I realize that when we start talking about plants, a master seed is not a very good term for you to use. Our master seeds and master cells are usually identified as a single lot with the identity and purity confirmed. And then all product is made from that master seed. I really like the concept of perhaps a master plant bank, and perhaps we will go to that kind of terminology. I hadn't heard that terminology before this meeting. We've talked about master DNA to talk about the actual insert.

We want to make sure that our regulations allow the use of different hybrids and different F1 hybrids and different strains that can be used for different fields and different types of field conditions that you might find year to year without having to restart all of the license application and restart all of our testing. This has not been a concern for our conventional products, so we will have to deal with how we can best regulate these products and still have assurance that we have a stable product. Potency assay specificity will have an effect on how we can look at our master cell or master seed or master plant bank definite because if we have

a potency test that adequately looks at the antigen, characterizes it very well, perhaps at the molecular level, and provides both identity and quantity, that will help us in determining whether or not these master seeds must be characterized more fully when you change hybrids.

Purity requirements obviously may need to change for oral products. It may not make sense to have a requirement that all products are pure. Right now we do have a requirement that products are not contaminated.

However, I don't think it's unlikely that we will ask you to set standards for acceptable contamination levels for all of the different kinds of contaminants that you may find in the oral products: the mycotoxins, pesticides, herbicides, soil-borne contaminants, the naturally occurring plant toxins, and any other feed contaminants that may be there. And depending on the type of product you have, we may need to look at what kinds of contaminants may develop over the storage of the product, whether you would have fungus growth, et cetera, in those products. Safety tests for plant-based veterinary vaccines will include our traditional safety tests, which means that you will be asked to administer to the target host at recommended dosage and scheduling. And we will expect that there are large numbers of target animals that are tested prior to licensure.

Obviously, reversion to virulence studies will not be applicable to most of these types of products as they are for our conventional products, but overdose studies are important, and certainly the induction of tolerance needs to be addressed prior to licensure so that you know what happens if animals are overfed your product.

If the product is grown in fields, obviously, we'll have an additional concern, and that is the safety in non-target animals. And obviously, the conditions put in place by PPQ will continue to be enforced during the growth of the product for production. You have to recognize that pleotropic and epistatic effects could theoretically result in expression of harmful substances and for feed-based vaccines could possibly result in a requirement for additional clinical monitoring of animals used in safety tests; in other words, long-term tests that we may not have asked for with our conventional products with which we have a higher level of confidence.

Specific means whereby non-target animal safety will be addressed are under discussions at the moment, but you should understand that we have a very high concern for what might be happening to animals that are exposed on a continual basis in the environment.

Particularly if you're going to grow these vaccines in the same place year to year and we were going to have non-target animals that are exposed year to year to the same antigen, we think these effects must be addressed prior to licensure, and that means that we are probably going to ask you for some direct studies of a number of different species, insects and birds, small mammals, maybe fish if the antigen appears in water runoff, reptile, whatever we think is appropriate. So environmental concerns are definitely a big issue for those of you who intend to grow your product in the field, and you should be aware that that will be evaluated depending

on your antigen. In other words, if the antigen is well-characterized antigen and we have some experience with that antigen in some of these other species, the requirements for testing will not be as severe as if we have no experience with that antigen. And I'll talk more about risk analysis more at the end of the talk.

Potency assays must measure identity and amount of specific target antigen or antibody. And all hybrids must demonstrate no detectable difference in structural expression of the target epitope.

The various antigenic concentration must be established during licensure, during prelicensure, but obviously, you'll be able to blend various harvests to produce your serial of product if you would have high and low yields within a given season. The potency assays should be approved prior to starting your efficacy and field trials because we're going to go back to that information when you want to make changes, and we're going to go back to that information for dose. And if you don't have a good potency assay before you do your field trial, we don't have a good way of determining what level of antigen you fed to your animals during your efficacy trials. And the potency assay also must differentiate serials that are efficacious – in other words, have adequate antigenic mass to accomplish the claim that you make for your product -- from those that do not have that.

You also should establish the fate of material that doesn't meet your standards. We need to know what you will do with product should it not reach the potency that you have set for erial release.

You should realize that potency test for our products are carried out on final or bulk product, not on preprocessing product.m,

For potency assay architecture, those of you who are familiar with our industry realize we allow a wide range of different ways of looking at your final product. You can either use in vivo assays with vaccination challenge or vaccination serology if you correlate the serological response to protection, or you can use in vitro assays, and those of you in our industry realize there's a lot of documentation available to you on what is an acceptable in vitro assay for us. VS Memo 800.90, which you can get on our Website, or 9 CFR 113.825 will tell you our requirements for in vitro assays.

But the basics are that they must be based on a protective immunogen, and you must test for relative antigen content and compare that to your original efficacy serial, either through a valid reference or some other method of tying it back to your original efficacy trials. And you should include an identity step in your potency trial for these types of products.

Efficacy requirements will be the same as those for conventional products. They will be based on label claims, and here are four of our typical biologics veterinary biologics claims: prevents infection with a certain microorganism, for prevention of disease due to a certain microorganism, as an aid in the prevention of a disease, or as an aid in the reduction of a

disease. These are the types of biologics claims. Your data must support whatever claim you intend to make on your product. It does require vaccination challenge of fully susceptible host animals, and they should be conducted on the youngest age for which the product is recommended. Sufficient numbers of animals must be at that youngest age, although not all animals need be at the youngest age recommended.

We do ask that you vaccinate and challenge animals that are fully susceptible. That, of course, doesn't deal with the issue of what happens with maternal antibody. We've heard some about that, but this is our requirement to demonstrate that your product will induce a protective immune response in animals that have not been vaccinated previously.

If maternal antibody interferes with your product, you should determine that prior to licensure, and you should make appropriate recommendations for animals that may be young enough to still have maternal antibody interfering with their immune response.

We ask that you give us at least two months to look at your protocols for efficacy and safety trial, and we will give you an evaluation of that trial and tell you if we would like changes in that efficacy trial before you start.

Label claims have been an item of much discussion in our agency for these types of products. 9 CFR 112 will tell you the conventional products requirements for labeling.

They do require directions for use, which includes dosage, frequency of administration, how to administer the product, and the minimum age at which you should administer the product to the animal.

Claims for protection must be on the label. Storage conditions and expiration date are important, and warnings, cautions, and restrictions must be on all of your labels.

Growth in fields obviously brings a lot of concerns for us. First and foremost are the standards for isolation and containment. The growth must be under the firm's control. And regulatory oversight or the right to inspect farm operations will be necessary, as will be the ability to follow the product from seed to final product.

So we are going to need very good records that document how much was planted, how much was harvested, and what happened to that product all along the line so we know that that product cannot and will not get into the human food or animal food chain where it does not belong.

If you use tissue-specific promotors, you may reduce some of those concerns. Certainly getting rid of the rest of the plant materials may change requirements if you have tissue-specific promotors.

We've had a lot of discussions about feed mills. Are these going to be licensed establish -- or what part of the licensed facility? And that answer is not definite at this time.

We will have the right to inspect, but we might not require that feed mills be part of the licensed facilities. That does not mean, however, that you won't have to identify the field mill prior to use of that field mill and that we might not inspect that field mill and ensure that we can preserve the identity -- that they can preserve identity throughout the process and that we can be assured that your product when it comes into the feed mill will stay unique. It will not be contaminated with other product nor will it contaminate other product. It's a very high concern for us since feed mills may also be processing food intended for animal or human consumption.

You might be able to do that even through dedicating the facility at the line at the time of production or dedicating the facility at the time of production and only dealing with that particular product. We will consider other options, but it's going to be important that we have assurance that that product stays identity preserved throughout the process.

Some constructs may not be eligible for growth out in fields, and that has to do with he toxicity of the antigen expressed or of the plant expressing the antigen. So it's important for you to think about that when you're looking at some of the constructs you may be using. If such a situation exists, then obviously, greenhouse growth is the way that that product will have to be grown.

We've had a lot of talk about what constitutes a serial for these products. In our traditional products, a serial is a homogenous product, and we can do serial release testing on one or two bottles, and that is representative for that serial.

Obviously, that's not going to be as easy with some of these types of products, and we may allow testing -- a statistically relevant testing of various time periods throughout the processing to take various samples.

Since milling and blending obviously don't yield homogenous products, we will have to deal with that on a product-by-product basis. Also, repackaging, reblending, and reprocessing rules haven't been developed at this time.

There are some documents available for plant-based veterinary biologics, but at this time they're under revision, and so if you check our Website in several months, we should have these available for you, and if you're working on products, I would encourage you to get in touch with us, and we will let you know as soon as they are available. And if you're going forth with a product, we would like to assign a reviewer to you, and that reviewer will work with you on our requirements and telling you where our current thinking is.

We have a document called a Summary Information Format that we use for all of our biotechnology products. That is simply a format in which we tell you how to present your

information to us, and we have a draft for plant-based products. Well, I should say actually for transgenic plants, not for the virus plant-based products, virus-vectored plant-based products at this time. We will have to develop that SIF also.

It does include the molecular characterization, and it does include a number of details that we need in order to do a risk assessment. The risk assessment will be a joint PPQ-CVB document, and we will be ensuring that non-target animal exposure is covered as part of that risk assessment. We will also have a draft outline of production guide for you very soon, and we have one now, but we will adapt that very soon.

I'd just like to talk for a moment about risk assessment. Risk analysis for us includes risk assessment, risk communication, and risk management. Nothing is risk-free in life, but we do need to do a risk-benefit analysis.

Risk assessment includes risk to animals, both target and non-target, risk to the public health, the human health, and risk to the environment. And that includes cumulative impact, particularly in places where the same field will be used year to year to grow this product.

It is then up to us to consider alternative actions that we have available, and basically for us, that's usually to allow or not to allow this to go to licensure and to be grown as proposed. We may ask you to modify the requirements for containment that you have proposed in order to meet our risk needs.

Risk communication happens when we publish your risk assessment in the Federal Register. All of our products have their risk assessment published in the Federal Register prior to licensure, prior to large-scale testing. And we do give a period of time for comments, and we will consider comments we get from the public prior to permitting the continued growth of that article for biologics production.

Risk management will include plans for mitigation of adverse events. In other words, you need to know what you will do if you have an adverse event in the field during growth. When we characterize risk, we look at the likelihood of an adverse reaction rating, whether it's unlikely, it might happen, or it most probably will occur, the consequences should that adverse reaction occur. Is it not severe, moderately severe, or severe? And the degree of certainty with which we make that evaluation.

Certain means that we have whether we have certain direct scientific evidence -- that means we've seen your antigen in the field -- whether we have moderately certain degree of certainty, which means indirect scientific evidence, or whether we're unacceptable, whether we have no scientific evidence, and all we're doing is making an educated guess.

The expected risk results in a risk rating, the likelihood times the certainty and the consequences times the certainty. And then we have a risk rating of qualitative, low, medium or high.

Next will be a request to ship an experimental biologic, and this is governed under 20 9 CFR 103.3. Now, those of you who are producing vaccine in the field, if you are shipping that for animal testing, you are in violation of our Act if you are not getting permission from us to ship that product.

So you should be contacting us. Once you have grown your product, if you bring it back to your facility, we are not asking you to tell us about that event at this point. We do have authority to ask for that information and for us to give you permission.

However, if you're going to take it off your facility's premises, then -- and these are unlicensed facilities as well as licensed facilities -- and you're going to ship it and that means that you're going to ship it across the street to another place that is not your licensed facility or your unlicensed facility, that is still shipping, whether you hand-carry it or ship it. You need our permission for movement. So you should be in contact with us.

Before we ship an experimental, we need to know what the risk of shipping that experimental. We do not ask necessarily for all of the details, but we need to know what your master seed is. What is this product that you intend to ship? What is the molecular characterization that you have? What hazard identification do you have? What's the risk characterization of moving that product?

If it's low, we have an acceptable risk. We'll approve the request. If it's medium with an unacceptable risk, we'll ask for satisfactory mitigation before we approve, and if we don't have satisfactory mitigation, we won't approve. And if it's a high unacceptable risk, we will not approve shipment.

Now, you're not likely to fall into any of those categories except the low acceptable risk, but it's important for you to recognize that you should be in contact with us, and you should be getting authority, and that authority will involve informing the state veterinarian of your intent to move and getting permission from the state veterinarian to move product into or within his boundaries that he is responsible for because this is a vaccine at that point. Prior to feeding an animal, it is a vaccine.

So in summary, the regulatory philosophy applied to conventional and existing products will be applied to plant-based biologics.

We will continue to harmonize our standards, and I think this meeting has set the tone for us to go forward to develop a harmonized framework for those requirements. And we'll continue working with the other agencies.

We also have a licensing packet. For those of you who are unfamiliar with our requirements, there are licensing packets that are available at the desk, and if you are seriously

considering production of a veterinary biologic, I encourage you to pick up a licensing packet and to look through it and see what our requirements are.

We would be happy to talk to you at any time and to assign you a reviewer at any time that you believe that you are going to go forth with an application for a veterinary biologic.

Thank you.

(Off-the-record discussion.)

MIKE PIONTKOWSKI: Mike Piontkowski with Colorado Serum Company. With plant-based biologics and specifically seed-based biologics, for instance, the ProdiGene product mentioned earlier this morning, is there going to be some sort of requirement that the product is in a form, a final form, where it cannot be propagated in the field again?

And the reason why I ask this is that we're dealing with seed-based product. Let me back up. With biologics, let's say, for instance, modified live bacterial product, obviously, Joe Farmer doesn't have a fermenter at home that he can grow this stuff, but a seed-based product, I would think that they could propagate on their own and then once it starts going down this road. Is that something that you're going to consider?

LOUISE HENDERSON: That would certainly be in violation of our Act for somebody to do that, so we will -- to propagate a vaccine without our permission.

I can't tell you what the requirement will be at this point, and I do not know that there will be a requirement that it not be capable of reproduction. We just don't have an answer for that at this point. It's certainly under consideration.

MIKE PIONTKOWSKI: If I was Murphy Farms and I had to pay 20 bucks a bushel for TGE corn and I had a planter and a cultivator and a combine, I sure wouldn't consider it.

LOUISE HENDERSON: I'm sure the firms that are producing this material are well aware of that danger to their product.

YASMIN THANAVALA: So excuse this ignorant question from a city girl, but I can understand how you would regulate dosage administration for a human vaccine like we gave these potatoes in little bags and watched people eating.

How do you envision regulating dosage for a farm animal? They're also feeding all together.

LOUISE HENDERSON: Yes, and that has been addressed, but we do have oral products already, and basically, for farm animals it's very different than for humans. We're really looking for herd immunity.

And these tests will have to be done during prelicensure. You're establishing what dosage per animal, and you feed that amount. You do not have a known dose that each animal actually ingests, but you should be achieving herd immunity with that dosage.

All I can say is that it's a very different type of immunity that's necessary when you start looking at most of these diseases. If you establish a herd immunity, the disease will not come into the herd.

DON REYNOLDS: Don Reynolds from Iowa State University, and I have two questions for you.

First, in terms of your labeling, I saw nothing on duration of immunity. Was this not a consideration?

LOUISE HENDERSON: Duration of immunity is definitely a consideration, and for all new products we are asking duration of immunity.

Now, I hesitate to say too much about that because that's very much a disease-driven event. In other words, if the disease is for baby pigs and they grow out of the ability to succumb to disease at a certain age, then duration of immunity obviously has a very different meaning than for those products that are for diseases that can affect an animal throughout its life span.

But yes, for other products we do ask duration of immunity, and we ask that that be established prior to licensure with challenge data at the end point.

DON REYNOLDS: Second question relates to the first gentleman who was questioning regarding Murphy Farms. What are your thoughts in regulating autogenous product?

LOUISE HENDERSON: I don't see that autogenous products for plant-based products will be any different from a combination of our autogenous products as we deal with them presently and the plant-based biologics requirements.

I really can't say much about it because to tell you the truth, we haven't dealt a lot with that kind of product yet, and I don't know what the likelihood is that we would be getting autogenous products for plant-based --plant-based autogenous products.

JOE JILKA: Joe Jilka from ProdiGene. I'd just like to address a couple of those questions, you know, as far as Murphy Farms and autogenous in that the product for all variety of reasons, whether it's intellectual property and regulatory, will always be delivered as a milled product. It will never be delivered as a viable whole seed so that there will be no possibility that that product can be taken out of the bag and planted.

DAVID ESPESETH: Other questions?

Yes.

JOHN THOMAS: John Thomas with Lumen, Lange & Wheeler. Dr. Henderson, it sounds like for the experimental stages and the plant production that the Center will be going out to the fields to be worrying about the environmental concerns rather than PPQ doing that.

Is that what is going to happen, is that you're going to use your broader authority than the narrow PPQ authority?

LOUISE HENDERSON: PPQ authority will continue to exist. We will have the right to inspect production sites. We have not established at what point we would be doing inspections, but we are trying to work very hard to ensure that we're using tax money wise, and we would not be duplicating efforts.

We would be working with PPQ to establish who would be doing inspections. Right now PPQ has a great deal of expertise that we do not have, and we would be relying on their expertise and their authority.

Our authority has more to do with control of the product. We might end up going to a field if we had some concerns about how much was planted and how much was harvested if we felt that we were not getting a complete picture.

JIM WHITE: Louise, mention that we went together last year.

LOUISE HENDERSON: Yes. Actually, we went together on a field trip last year. Our entire coordinator review team was hosted by --Jim White had arranged for us to have an inspection with PPQ investigators, and we went with them to see how they investigated and how they inspected fields.

However, I'll repeat. We hope not to duplicate those services. It's very expensive to go on site for inspections, and we hope that we can have whichever inspectors go meet both PPQ and CVB needs. They are all APHIS employees.

TOD STOLTZ: I'm Tod Stoltz from Applied Phytologics. You mention that there will be some draft guidance documents available shortly. Will those documents include guidance for little companies like ours on how these transgenics should be generated in the first part; for example, microbiology systems?

Do you have any backbone DNA in the plants, or should we try to remove that? What selectable markers are acceptable to use, which aren't, information like that?

LOUISE HENDERSON: Yes, they will have some of that information. However, we would be happy to speak with you individually about what you are thinking about using and telling you where our current thought is on the use of markers, especially if that's an important consideration.

I would urge you to be very cautious about use of antibiotic markers in your plants. That's obviously a very big issue for animal use.

Thank you.

DAVID ESPESETH: Our next speaker will be talking about compliance with current good manufacturing practices, and he's from the Center for Biologics Evaluation and Research.

Michael Brennan is with FDA-CBER. He received his Ph.D. from Albany Medical College in New York in human experimental pathology, and also, he completed a post-doc at LaJolla Cancer Research Foundation in LaJolla, California. He joined NIH in 1983 as a staff fellow in the microbiology laboratory at NIDR, NIH and in 1986 he joined CBER working on acellular vaccines for whooping cough and in 1992 joined the laboratory for mycobacterial diseases where he currently is conducting research focused on the pathogenesis of tuberculosis. He also is responsible for the review of license applications for new vaccines and diagnostics for tuberculosis.

So Dr. Brennan.

MICHAEL BRENNAN: Thank you. Bill Gates was our low bidder, so I got PowerPoint too. So three regulatory talks in a row just before lunch, so if we were at a different seminar, I'd ask you to get up and do breathing exercises.

But anyway, my charge today is to share with you an overview of the federal regulations that are followed by manufacturers who produce human biologics for licensure in the United States.

I briefly want to discuss four things that I'm going to try to cover: one, go through the major current good manufacturing practices as outlined in the Code of Federal Regulations; secondly, indicate where a more thorough discussion can be obtained of the manufacturing practices in the CFR, the Code of Federal Regulations, or in FDA guidelines.

I'll address some of the specific manufacturing issues that we've been discussing in our working group with Jim White and Louise Henderson and others about how we're going to apply these regulations to transgenic plants and, also, at the end, discuss just a few items about testing edible plant vaccines in both preclinical and clinical trials. I know this is really not GMP,

but it's something that brings up some of the more interesting questions as far as how we're going to regulate these products.

I'm going to ask a few questions in my slides. And I'm doing that so you don't ask me at the end. That helps me out. And the overall goal, as you know, of this meeting is to try to develop guidelines, regulatory guidelines, so that we'll be able to better manufacture these products in a safe manner.

Just one slide about the Center for Biologics Evaluation and Research, which is located in Bethesda, Maryland. Many of us are researcher reviewers. As you heard, I'm presently in the laboratory of mycobacterial diseases doing research on DNA vaccines for TB. So many of us have active programs in the pathogenesis of infectious diseases as well as review work. These are some of the products that the Center for Biologics reviews and licenses including the vaccines which would fall under many of the topics we've been discussing here and therapeutics and monoclonal antibodies.

Dr. Henderson showed this slide already. This gives us the authority for regulating biological products, so I won't go through that in any detail.

The bottom line, I think, for licensure is stated here. Basically, it's our charge to put on the market safe and pure and potent and efficacious products. And the way to do that is by the implementation of good manufacturing practices. And that's why we have these good manufacturing practices.

This is kind of the guts of the talk here, and I'm going to spend a little time on this. Basically, these are the subheadings found in the 21 CFR Part 211. These are all the subheadings under the current good manufacturing practices, and there's a more thorough description under each of these subheadings. I'll just touch on a couple of topics that are contained in these subheadings. In organization and personnel in this first section, this is a discussion of the organization of the manufacturing operation itself. It includes a description of the quality assurance programs and how the quality assurance programs which maintain the batch production records are done. It oversees the quality control testing at all stages of manufacturing. This section also talks about he training that's required for all the manufacturing staff and the QC staff.

The second heading is building and facilities. This focuses on all the air and water systems in the stand-alone facilities on animal facilities.

The third is equipment. This talks about the validation of all equipment that's used in the manufacturing, including the GBRTs we heard about yesterday, lyophilizers, fermenters, freezers, incubators, glass vial washers, any type of equipment. It includes the cleaning and maintenance of this equipment as well.

The fourth, is the control of components, containers, and closures, this points out the need to control all components that are used in the manufacturing process, including raw materials, and points to the importance of the quality and the cleaning procedures for containers that hold the biologic.

Production and process control, this describes the methods needed to test for microbiological contamination. Time limitations between processing steps, mixing procedures, pH, reprocessing of batches, all of those types of in-process tests.

Packaging and labeling control, this points to the importance of physical separation of unlabeled and labeled product, primarily to preventing mislabeling of product and also talks about expiration dating. And the holding and distribution, this is about warehousing and distribution procedures.

Laboratory controls, this describes final product testing, including tests for identity and sterility and stability. Records and reports talks about the importance of documentation, including keeping records, all production records or the BPRs, records for quality control and for distribution, and also includes the keeping of equipment logs and complaint records from the consumers.

And last is the area of returned and salvaged products, which talks about reprocessing and having to do investigations for any products that are returned.

This is actually the motto of FDA's Office of Compliance, and I think this statement basically stresses the importance of in-process testing. What it says, the bottom line here is that filter sterilization of a final product is not going to be a remedy for excessive bioburden during production, and the philosophy is that all steps in manufacturing are important.

In the case in your airport bookstore you see a copy of the CFR, you could look at these sections on the way home. And what I just talked about in Parts 210 and 211 describes the current good manufacturing practices. The other part that might be most important for what we've been discussing are Part 600 which talks about biologics specifically. Other parts that may be applicable are Part 25, which discusses environmental assessment; 225, which are GMPs for medicated feed; and Part 820 if you were developing a product in plants that might be used in a device. An example might be protein kits that might be used for an ELISA diagnostic kit where the protein was made in plants.

I think this is the primary question we're all here to address when it comes to manufacturing. Can agricultural facilities that produce plant-derived biologics maintain the appropriate good manufacturing practices required by the FDA for the manufacture of biologics for human use?

And I think to be honest, the FDA is used to applying regulations to manufacturing plants but not to plants used for manufacturing. So a lot of this is new to us as well, and that's why I won't be able to answer any questions at the end!

Dr. Henderson talked a little about what is on this slide. I'm just going to go through a few of the good manufacturing items in a little more detail at this point. The FDA requires a complete history of the origin of the biologic that's used to produce the finished biological product. For example, for bacterial vaccines --like the tetanus vaccine which contains a purified tetanus toxoid component -- there has to be complete characterization of the clostridial strain that's used to cultivate and purify the tetanus toxin protein.

This includes a history of all the subcultures and the storage and transport. It includes showing that this original culture has been securely stored under recorded temperature conditions and that there's been periodic testing for viability and identity. By the way, that's contained in the CFR, both in the 211s and the 610s.

The question for these new transgenic plants is, how can viability and composition of the seed stocks be maintained during long-term storage for both inbred and hybrid transgenic plants? And I think we heard yesterday from Dr. Russell some good evidence on how we can start characterizing a primary corn seed, and again, this gives renewed meaning to the term seed stock here as Dr. Henderson mentioned, and I think maybe what's even a little more complicated is characterization of systems like Dr. Holtz talked about, the viral particle systems. What is really going to be the original seed stock here? Are we talking about the DNA plasmid? What's going to be needed for characterization of the viral particles, and what about the host plant? How are we going to characterize the host plant as well?

So some of these are complex issues that are going to have to be discussed, and maybe discussions like that can continue in this afternoon's round table.

As far as the recombinant product development, a number of issues have already been outlined in two guidelines that are points to consider documents from the FD that can be obtained on the FDA Website on plasmid DNA vaccines and the testing on monoclonal antibody products.

There are parts in here that are applicable to both the recombinant steps that occur in the viral particle approach and in the Agrobacterium approach, but there are also some other points to consider documents that you canfind on the Website page that I haven't put up here for the sake of space that may also have applicable sections in them.

So some questions about transgenic plants occur here. We've heard some talk yesterday about the possible use of inducible promotors, tissue-specific promotors or auxotrophic mutations in transgenic plants. These would address environmental concerns, especially about containment, so these might be good things.

Secondly, should transgenic plants carry distinguishing markers such as distinguishing color? We heard about the white tomato, and this may address issues of identity, especially in the final product. So in plant propagation, which is the stage of manufacture that would be like fermentation of a bacteria in the example I gave before that is the tetanus vaccine. For the tetanus vaccine, the containment vessel would be the fermenter, and the raw materials would be the culture media.

There would be routine sampling to test for viability and bioburden, and there would be control of additives such as the anti-foaming agents that are added to fermentation.

In the plants we have a similar situation except we're talking here about the issue of field containment. We're talking about the addition of pesticides and herbicides and topics like should the use of pesticides be restricted at a certain time before harvest, for example?

The media is the soil and the water, and there's issues like heavy metals and sewage treatment and fermentation. And then there's the in-process testing during plant propagation, especially for organisms like molds that may contain toxins.

After propagation we come to the stage where we have to start discriminating between the intended use of the transgenic plant. If it's a biologic that's going to be purified from the plant, it will be treated much like our more traditional biologics for testing from here on out.

The unique regulatory control methods, though, will come for the edible biologic vectors. And I'm going to talk more about that. Bulk Parts 211 and 600 have a thorough description of the product testing that needs to occur at this stage since in some cases the product at this stage can be the licensed product.

Some questions arise, for instance, what requirements should be instituted for the disposal of residue? We heard some discussion about that already. It's possible, though I don't know -- I think Jim White knows a lot more about this than I do -- is that there could be proposed multiple use transgenic plants including both product and silage. How do we deal with that? Can we use residue for feed or for fuel, for example?

And I think there's going to be more discussion of this this afternoon at the round table as seen on the sheet that Dr. Price has composed. The CFR 610 contains requirements for all this type of testing on the products. How are we going to do this type of testing for transgenic plants? Product sterility is probably going to equate with bioburden for plant-derived biologics, and as I mentioned, molds may be especially important.

This part is also interesting. How are we going to characterize the products that are made in plants? We've heard discussions of glycosylation being different on the antibodies. If

we wanted to make a protein in plants such as the OSP-A protein that's used for Lyme disease which is lipidated, can we do that? Is that a possibility, and how will we characterize the lipid?

And protein conformation, we've already seen evidence that the LT-B can form the right conformation in plants and monoclonal antibodies can do this as well. And there are other examples, but this is going to be important entity if we're going to look at the development of new vaccines in plants, for instance, such as the toxin vaccine that need to oligomerize into the correct form.

Okay. From here for the next three slides, I'd like to address the issues that have to do with the edible vaccine products. For biologics that are purified from plants, the final testing will be similar to the more traditional products.

The testing for the edible vaccine products may be more complex, as most of you realize. These are the final product tests that are required in Parts 211 and 210 of the CFR, and for instance, take identity, if we're talking about a tomato, how are we going to distinguish that tomato from one which is not expressing the protein of interest? For sterility we've talked about bioburden, but what are we going to compare that to? Are we going to compare that to store-bought fruits or vegetables which we heard about yesterday, whichmay contain large amounts of micro-contamination?

And stability, here we have to be concerned with expiration dating, and how are we going to determine expiration dating? We're used to dealing with products that have expiration dates of one to two years or more. These products will be quite different.

An issue that Dr. Mason brought up in his talk today is this one about dose in plants. What tests are we going to have to measure lot-to-lot consistency for edible vaccines, and how are we going to know what dose we're actually giving in the final product? There are ideas out there that we've heard, such as from Dr. Richter yesterday, using either baby food jars of food or puree or juices which would then give us a more batch approach to looking at this dose question in edible vaccines.

Prior to going into humans, there's commonly animal work done, at lease a certain degree animal work done. Some tests are required which includes safety and toxicity testing, but again, our current requirements for safety suggest that standard amounts of the product be given IP to both mice and guinea pigs. And so this test will probably need to be amended for edible vaccines because it won't be a viable test for that kind of a vaccine. Often the animals are also used to develop potency tests and to develop an animal model for the disease to give efficacy and effectiveness information.

We've heard talk about some animal models for plants already. Commonly the mouse or rat will eat most foods, and in our lab, for instance, we've been doing some work with Dr. Levikoff and Dr. Keith who have two posters here, and we know that the mice will eat

tomatoes, and you can elicit an antibody response . But other animals can be used such as out here in Ames, we may want to use this animal here (pig).

The final issue relates to clinical trials. And there's a lot of interesting issues here as well that we're going to have to deal with that Dr. Mason summarized this morning, including the ones about dose and dose regimen. These will have to be worked out well in the Phase 1 trials, and in Phase 2 human trials.

There are particular safety issues for transgenic plants including plant toxins, allergic responses, and immune tolerance. The efficacy trials will require a type of immunization center to deliver these edible vaccines. And from the picture presented this morning, it looks like they'll have to be equipped with potato peelers.

And then there may have to be more post-marketing surveillance than usual because if this is given as a food, there's going to be certain efficacy and long-term safety questions that might have to be addressed differently than other vaccines for which we just have more experience So post-marketing surveillance might be extended for these kinds of approaches.

And lastly, in order to obtain information, this should be in your book, there are sites that you can contact at FDA for more information on what I've been talking about, including a fax number, and an Internet number. At this Internet number the whole list of guidelines can be downloaded, I think, through Adobe Acrobat and other systems.

This is the Office of Public Affairs since I can never remember what the acronym means, and you can e-mail them for more information from FDA, or you can write to them here.

So thank you very much.

DAVID ESPESETH: We're now open for questions. Are there questions? Yes.

GREG BOBROWICZ: My name is Greg Bobrowicz. I'm an independent consultant. Just a comment and then a question. Comment is that of course, Part 211 is the good manufacturing practice regulations for the pharmaceuticals, and so for purified biologic it would not apply to the things we were talking about, and I think it would be a very interesting discussion one way or another to either apply it to edible vaccines or not. I'm saying it would be controversial. The question is, does FDA consider that their inspectional authority under 704 or the PHS act will extend to farming operations, and if so, who's going to do that biologics or --

MICHAEL BRENNAN: Could you repeat that?

GREG BOBROWICZ: Does FDA consider that their inspectional authority extends to the farms? And if so, who's going to do that, biologics or just field investigators?

MICHAEL BRENNAN: I think yes if it's a biologic, that was my main point of that slide, putting up the manufacturing plant in the field, was that if it's a biologic, we will have the authority to inspect.

It will likely be done by team biologics, but then the preapproval is often accompanied by the chairperson of the product from the Center for Biologics.

TOD STOLTZ: Tod Stoltz from Applied Phytologics. In the safety testing or the safety issues you mentioned for plant-derived biologics, you mentioned allergenicity testing and the response. Does the FDA have a model in mind for testing the allergenicity of biologics derived or delivered through food?

MICHAEL BRENNAN: No.

TOD STOLTZ: Are you considering --

MICHAEL BRENNAN: I don't mean to be abrupt.

TOD STOLTZ: Sure. I understand.

MICHAEL BRENNAN: No, I don't think we do. This is, you know, a question that was brought up by the FEMA situation that we're aware of. Those are things that will have to be addressed and worked on.

TOD STOLTZ: And how are those issues going to be addressed?

MICHAEL BRENNAN: This afternoon, I guess.

KATHRYN STEIN: We have somebody from CFSAN who will be on the panel.

A question for Dr. White. I don't know if we're going back to the other reviewer. Jim, you mentioned about these products will not be available for nonregulated status or deregulation biologics. Can you comment on the views going and using the current process when you go from what could be single acres not to hundreds of thousands of acres but what could be thousands of acres, and are there triggers you see then or thresholds, or how does this EA process change as you go forth?

JIM WHITE: Okay. I think it was clear, you know, Louise talked about --Dr. Henderson talked about NEPA compliance when they do an assessment at that point. So the agencies will work together, and we've agreed to work with the Food and Drug Administration on NEPA compliance and provide information for that.

I think the hard part about the amount of land needed, it all goes to how much product is produced, and it's too early to know. You know, obviously the concerns, you know, 500 acres or 50,000 acres are going to be quite different, and so that's probably -- it's all going to depend on market share and the expression level, that we're going to have to wait and see as the products come closer and closer to the market.

I was supposed to mention one other thing. This lady here who's doing the transcripts, the whole transcript of the whole meeting is going to be available in about six weeks at the FDA Website for downloading.

KATHRYN STEIN: It will be hopefully six weeks, but it will be available.

DAVID ESPESETH: Other questions for any of our speakers or Dr. Brennan?

I have one for APHIS. Considering the use of plants to produce veterinary biologics will mitigate a lot of concerns about foreign animal disease, I would think that these types of products could move in international markets without a lot of concern if they're pure, safe, potent, and effective.

Has there been any thoughts by the working group as to where they go from here, and will these regulatory requirements be discussed internationally? Because we talked about if we're going to harmonize, we should harmonize as these things are coming on-line rather than trying to get together after each of us have established something.

LOUISE HENDERSON: We have talked in the working group about this, but we have not at this point approached other national regulatory bodies about how we are going to regulate these products.

DAVID ESPESETH: Also, will the working group consider other inputs for public opinion to get consumer inputs in the process?

LOUISE HENDERSON: Certainly we would like any input we can get. That was one of the intents of having this meeting, and yes. I believe we will be going out and asking -- we will be publishing in the Federal Register when we are at that point and asking for comment, and we will also be providing documents on the Website and various other places and asking for comments from both the industry and from consumer groups.

DAVID ESPESETH: Well, if there are no other questions, we thank the speakers for the excellent presentations, and we'll let you know that there is going to be lunch served upstairs as there was yesterday, and you're all certainly invited and encouraged to participate in that.

We will see you back this afternoon at 1 o'clock for the open hearing where this will be open for comments from anyone who wishes to come forward.

(Lunch recess.)

DAVID ESPESETH: I think we should get started this afternoon. As you know, one of the parts of this meeting is the inclusion of an open public hearing to discuss the issues related to the registration requirements or licensing requirements for plant-derived biologics.

And so this portion of the meeting has been set aside for anyone who is interested and wants to make a comment or raise concerns or point out potential risks that need to be addressed by the regulatory agencies as they go forward in the development of regulatory requirements for this category of product to have an opportunity to present those to the panel that's working on developing guidelines.

This is an open discussion or an open opportunity for anyone who wishes to come forward and make a comment. We have three comments that we will or have been -- we've been advised of three people who would like to make comments and give us their prepared statements.

If there are other comments following that, we will take comments from the floor. However, anyone who wishes to comment, please come down and give them from the podium and identify yourself and then follow up your comment with written material.

We also will be open to receive written comments if you do not care to make a public oral comment to give us your written comments up until -- we had a date in the announcement. I think it's a couple weeks.

I think the seminar that we've had over the last day and a half provides some excellent background from which we can have some discussion. First we'll have our open public hearing, and that will be followed immediately by our round-table discussion where even though you may not want to make a comment about what the regulatory requirements might be for these products, you may have some further questions for some of the speakers or some of the issues raised during the seminar, and so that round table will be another opportunity for you to follow up on any additional issues or questions that you may have.

So at this time we would like to open our public hearing with our first speaker, and that will be Don Emlay from ProdiGene.

Don.

DON EMLAY: Thank you. My name is Don Emlay. I'm with ProdiGene out of College Station, Texas. I think that first I want to say that this has been a wonderful opportunity. I'm new to the wonderful world of biologics expressed in plants, and I think the opportunity to hear the comments and interact with a lot of the people here has been really a unique experience for me, and I think probably true for other people here as well. Rather than

reading this entire statement that is probably quite boring and it may go on forever, I will just enter it into the record and attempt a brief summary.

All the opinions and ideas included here represent a group of us at ProdiGene getting together and thinking about these new products. I was just elected to make the presentation.

Most all of the thoughts in the written statement have become retrospective as they are now simply a reiteration of what's already been stated by the previous presenters. I believe we have some very effective regulatory processes in place, and the regulation of these new products, be it an edible vaccine or a product that is purified from a plant source, fall under existing processes that have been proven over the years. This includes the more recent FDA policy of 1992 that addresses the safety of food and feed from transgenic plants and the USDA regulation of transgenic plants that addresses environmental safety.

Basically, there are groups within the FDA and USDA that have been regulating biologics and transgenic plants for a long time. It is now just a matter of utilizing these existing processes and being certain they come together in the right way.

My simplistic view of is that for edible biologics for animals or for humans, you apply the existing assessment procedures for plants and in the end if corn is corn, then those procedures that are used today to grow and process corn into animal feed or human food are really no different for edible biologics than they are for the food product. If nothing about the corn has changed, you only have to address that biologic. That's been done for a long time the agencies know how to do that.

So the statement that I'll hand in really just brings these thoughts together in a longer and hopefully more coherent version. It is evident all of these elements have been considered and discussed and it's clear that all the agencies and people are interacting.

So ProdiGene and I want to thank you for the opportunity to be here and to put these thoughts and statements on the record. Thanks.

DAVID ESPESETH: Our next comment will be from Kent McClure of the Animal Health Institute.

KENT McCLURE: Good afternoon. I want to say ditto to everything that Don just said, and that's basically what I had to present, but I won't be nearly as eloquent. But I'll try.

For those of you that aren't familiar with the Animal Health Institute, I want to tell you that we are a trade association representing manufacturers of veterinary pharmaceuticals and veterinary biological products; really, any animal health product.

The United States, as most of you know, has the largest animal health product market in the world, and by sales dollars, AHI member companies represent 95 percent of the domestic feed additive market. They represent 95 percent of the domestic feed additive market and 75 percent of the domestic veterinary pharmaceutical market.

And as an association, we are excited about this milestone in the development of this technology, and we're very excited to be involved in what we believe to be a very promising avenue for the improvement of animal and human health.

We believe that this technology will provide the practitioner and the produce with new delivery systems and with new production platforms that will compliment existing products. And I think that's a key.

Nobody expects this to be the panacea that does away with existing technology, but we believe it to be an exciting new complement to the products that are currently on the market.

We believe that plant-based biologics will have some unique advantageous attributes that the producer and practitioner can exploit for improved herd health and improved human health, the most notable of those being the elimination of a cold chain and the concomitant facility requirements.

We also believe that oral products will eliminate the need for special training in many circumstances. No longer do you have to train people in injection techniques or aseptic administration of products, and you don't necessarily need the requirement for needles and syringes.

The products will provide convenience in administration and, significantly for producers, will decrease animal stress due to handling. And for anyone who has worked cattle, I think as was stated previously, anyone who's worked with animals knows that there are definite production losses that are due to the repeated handling of them.

Plant-derived biologics would also allow greater and easier international movement of veterinary biologics due to the removal of concerns over the introduction of exotic agents of animal origin.

Plant-derived -- and we think this is an important one. Plant-derived products have been demonstrated to be safe with no known adverse health consequences in man or animal, although we will acknowledge and state that we need to continue to evaluate safety, and the companies developing these products are continuing to do so.

A tremendous advantage is the stability of proteins that are derived from plants and the long-term stability for storage that they offer.

And another significant one is that the products will offer consistent cost reductions at each step from production through use. Most exciting of the advantages is that the benefits will allow producers and practitioners all over the world -- will allow them to implement sound practices for disease prevention and control, regardless of their individual level of sophistication.

There remains a tremendous amount of work that needs to be done prior to the widespread commercialization of this technology. Everyone in this room understands that. And what we want to bring the message, that as an industry, we're committed to working cooperatively, in all aspects, with government, producers, practitioners, and consumers to responsibly develop this technology for the benefit of both human and animal health.

Thank you.

DAVID ESPESETH: Our next comment will be from Jeff Meis, Iowa Crop Improvement Association.

JEFF MEIS: Good afternoon. I appreciate the opportunity to speak to you today. What I'm going to talk about today is identity preserved programs for grain and products from plants.

A little background information. Iowa Crop Improvement Association has been designated by the State of Iowa as a 78-year history of seed certification. Our seed growers make up the membership of the association, and our mission is to provide an unbiased source of service and education in the production of quality assurance for Iowa agricultural crops.

Iowa Crop Improvement is a member of the Association of Official Seed Certifying Agencies. This organization, members are agencies that are responsible for seed certification in their respective areas. It was organized in 1919 under the name of International Crop Improvement Association and is composed of seed-certifying agencies in Canada and the United States. It also has a dozen other countries that have affiliation with this organization.

These agencies maintain a close working relationship with the seed industry, seed regulatory agencies, governmental agencies that are involved in international seed market development and movement of seed, also in the agricultural research and extension services.

One of the purposes of why AOSCA has brought this identity preserve program is to identify grain and plant products that have met specific genetic traits and to preserve the genetic or physical identity of these products.

Individually AOSCA agencies have long offered a variety of these identity preserve programs along with their seed certification programs. Examples of this would be field inspection, lab-touring inspection, inspection of segregation of soybeans and so forth that are destined for the food-grade market.

We've also had services applied to high amylose corn and high oil corn. In general the IP programs have assured customers of the presence of those traits and conditions. The identity preserve program that we offer a result of a recent initiative has kind of brought about -- we're using it with the certification process. With certification we keep detailed record-keeping. This traces back to the seed stock and foundation stock of seed, precise field inspection protocols, accurate laboratory analysis, and testing and also official labeling.

We have been involved with Monsanto when they had brought out the RoundUp Ready soybean. They were wanting a program for quality assurance. We then went to them, received some protocols. Seed companies wanted us to be involved in this because of their licensed agreements. They had to have a quality assurance program in place. The AOSCA agencies offered these new services to meet the new demands.

With the advent of introduction of biotechnology in agriculture, customer demand for AOSCA services has both broadened and intensified. We have learned from the past and are trying to better anticipate what will be expected of us in the future.

In recent years we observed a demand shift from seed certification to quality assurance programs and IP programs. Interest in selecting against certain traits has emerged and supplements the desire to select for specific traits. Along with increasing and shifting service demands, AOSCA agencies have recognized the importance of achieving harmonization and standardization of their services for the food and agricultural industry.

The evolution within AOSCA that led to the development of a coordinated approach of offering identity preserve program began with the formation of an ad hoc committee focused upon the need for standardization of these services.

It was then taken forward. A certification trademark for identity preserve logo has been designed, and it has been filed with the U.S. Patent Office. Standards for the use of the logo were developed following these standards format utilized by AOSCA agencies.

The standards that are included in this program are eligibility requirements which we are looking for descriptions of what we need to look for in the field and in the lab.

Applicants' responsibility, which is the responsibility of making sure the planters, harvest combines, and so forth are clean, application for field inspection, establishing a source of seed and field inspection, which is also included as reports, field inspection reports, product handling, transfer of product to labeling, and also the carryover product and also some of the labeling to be sent out.

The general requirements will govern all future uses of the logo as a label. New programs will be developed for the industry as interest warrants. The general guidelines also allow provisions to the IP processes used as well as products produced.

What will the future hold for AOSCA identity preserve programs? Clients will be assured specific traits are maintained in all production processes. This will be even more critically acclaimed as high value traits enter the marketplace.

The logo will become a recognized symbol that the IP program has been implemented and overseen by Association of Official Seed Certifying Agencies. AOSCA agencies have years of experience in designing and delivering quality assurance programs. Some of the states that do have IP programs, some of them have been doing it for about 10 years. They also have labs to do the different testing and so forth for grain quality. The partnering of buyers and seller to select third-party identity preserve programs offered through AOSCA agencies makes good business sense. What we have in our program, it's more of a system approach which we feel is more meaningful, more practical, and more cost-effective than an approach just solely based on testing alone.

For a statistical vantage point, one can also expect the IP system approach in combination with other minimum product testing to achieve a high degree of probability that seed or grain identified has been preserved.

We invite you as industry leaders to partner with the AOSCA agencies to meet your customer needs. AOSCA pledges its collective integrity and technical understanding to assist with trade activities involving U.S. agriculture products.

Thank you.

DAVID ESPESETH: Does anyone else have a comment that they would wish to make at this time?

If not, we thank you very much, and this concludes our public hearing for the presentation of comments, and we will now proceed with the round table.

Speakers, please come down.

BILL PRICE: Looks like everybody is in place. I'm Bill Price from FDA Center for Veterinary Medicine. And one wonders, why does Center for Veterinary Medicine get involved?

It's because I'm an animal feed person, and remnants from these plants and the residue from the processing of the plants could possibly be used for animal feed, particularly if you get large quantities of it.

So in doing all of this -- Can everybody see the module? That's the regulatory molecule. And in order to detect this regulatory molecule, you need a special technique called World Wide Web, and that will lead you to various parts of the molecule.

I think one of the things I wanted to speak to initially was the acronyms – all right? -- that you will note up there. You have USDA-APHIS-PPQ, which is certainly the United States Department of Agriculture, Animal and Plant Health Inspection Service in the Plant Protection and Quarantine.

Then you have USDA-APHIS-CVB, United States Department of Agriculture, Animal and Plant Health Inspection Service, Center for Veterinary Biologics. Then you'll have FDA-CBER, United States Food and Drug Administration, Center for Biologics, Evaluation, and Research.

Then you have FDA-CVM, United States Food and Drug Administration, Center for Veterinary Medicine. Then you'll have FDA-CFSAN, United States Food and Drug Administration, Center for Food Safety and Applied Nutrition. And then you'll have NIH, which is United States National Institute of Health.

Underneath that you'll see these different acts. Now, the acts are congressional mandates. It's our commandments and that's what we work from. So we have the PP Act, Plant Pest Act. We have the PQ Act, which is the Plant Quarantine Act. We have the FD&C Act, which is the Federal Food Drug and Cosmetic Act. We have the PHS Act, Public Health Service Act, and the VST Act, which is the Virus-Serum-Toxin Act.

Now, the question is, how do you get from an act to the actual working to how the government regulates? We have the Federal Register. All the workings of the government on a daily basis are published in the Federal Register.

So if you want to know what's going on with the government each day, you get the Federal Register, and you read it. However, you might not be able to read the total thing every day.

So each of the agencies has their own part in the Federal Register which they codify, and there are different titles, and I think APHIS works under Title 7. Food and Drug works under Title 21.

Now, within these titles covers all different agencies within that title. Like with Food and Drug, you'll have parts that deal with foods, like Part 100. You'll have parts that deal with drugs, and you'll have parts that deal with biologics and parts that deal with veterinary drugs and animal feeds. So each part deals with a different section of that agency.

Then after you have regulation, it's probably not enough direction for that agency to carry on their total business. So then you'll have guidance. There are different types of guidance. I can speak mostly of Food and Drug Administration because we have things called compliance policy guides and compliance programs, which tells us how that we're going to regulate a certain program, and if there are specific points within that program that we regulate, we'll put out a guide for that.

When we establish those guides and make them public, we'll usually put a notice in the Federal Register called a Notice of Availability, and within that that will tell us how we're going to regulate a program or a particular section of a program.

Then in addition to that, we may do additional guidance for guidelines for industry. Then usually I think our tendency now is to also publish a Notice of Availability in the Federal Register when we do those. Now, those records are kept within the various agencies then. With the Federal Register, of course, that's available to anybody that wants to buy it from the documents part of the government.

And again, all these things are more and more we're having them on our Websites. So if you go to the various agencies, various centers within the agencies, go to the Websites. Pull up a given topic, and you can find most all of the guidance.

You can find the Federal Register documents that are current and pertain and so that you can work your way around through the Web and find what you're looking for and find the guidance that you're looking for. So that's what that is all about.

In looking back at the diagram now -- let's see if I can do this right. Okay. Now, I took the first box from there, enlarged it, and this is Jim White's territory. If you're lucky, you'll find a contact person that -- you find two contact persons: one for import of veterinary biologic products and one for permission to ship, and that's in the handout. Everybody has that. So those give the contact people for doing imports and provision to ship for this part of the diagram.

Okay. This is going into the program, I think. Okay. Okay. So work around all the acts that they work under, the PP Act and the PQ Act and NIH guidelines for containment. All right. Now, I go back here. We'll pick up the middle left big box there. So human biologics go to CBER, preclinical shipment preclinical and clinical trial review, licensing working under the FD&C Act and the Public Health Service Act.

Okay. Then we go to manufacturing, still under CBER working under Food and Drug Cosmetic Act and the Public Health Service Act.

Okay. Let's go back. Okay. Then we go -- which will be to the Center for Animal Biologics now. We'll go back, and we'll pick up the one that deals with what CVB does by themselves. So they do preclinical shipment, preclinical and clinical trial review.

I think this has gone into this program that I had set up for it. And then it goes licensing, and it's all under the VST Act. Then you have the manufacturing is also under the VST Act.

All right. Let's go back up to our diagram. Okay. Now we're going to add onto the animal biologics boxes, which is the box dealing with the combination animal drug. Okay. Combination with animal drug and feed and labeling. So we have with animal biologics what could happen, and we're only envisioning this, now, that if you were doing a biologic that also contained an animal drug, then it would be a combination, and then there would be two centers involved, would be the Center for Veterinary Biologics and the Center for Veterinary Medicine.

Also with feed labeling. That way we have Center for Veterinary Biologics, Center for Veterinary Medicine, and the states get involved with labeling. Each state has a labeling law, and each state has their own labeling requirements, though they work through a central organization called the Association of American Feed Control Officials to work out standard labeling for states, so -- and Center for Veterinary Medicine works closely with that association to make sure that everything works smoothly through that operation.

Okay. Now we're going next is to look at the disposal of the residue from manufacturing. So the residue, I think Dr. Brennan talked about the disposal of residue, and I think Dr. Henderson may have talked some about residue from the manufacturing. And so the residue for disposal other than food and feed is state and local laws for the most part. The residue for food would come under Center for Feed Safety and Applied Nutrition. We have Dr. Ditto is here from CFSAN.

And then residue for feed would be Center for Veterinary Medicine, and then that would be me. Okay.

Now we get to go to your far right on your diagram, and you'll see what's happening with the remnants of the plant that are not used in the process for deriving the biologic. We get down to our last slide there.

So we're working down. You can get -- I think most always -- most always it would come through PPQ. We can discuss when it might not but most always comes from PPQ. If you do for compost, that would be under PPQ -- I mean the PP Act. For remnants for food, again, CFSAN, and for remnants for animal feed would be CVM.

Okay. I think at this time can open up the floor for discussion.

DAVID ESPESETH: Do you have any questions for Dr. Price on his presentation? If not, just to lay some ground rules for this round table, it's open for speakers to ask speakers questions. I'm sure that you've also been in the audience and may have some questions that relate to some of your colleagues that presented. So don't be hesitant about the speakers asking each other questions.

And also from the audience, if you have questions, if you direct them to a particular speaker, that will certainly facilitate.

Before we start, it might be good, we have the time for each of the speakers to go through and introduce themselves again just so everybody is aware of who's here. And so if we could start with our first speaker. Just name and --

GUY CARDINEAU: I'm Guy Cardineau. I'm responsible for output agriculture for Dow AgroScience.

HUGH MASON: I'm Hugh Mason at Boyce Thompson Institute at Cornell working on plant vaccines for human and animal.

JIM WHITE: I'm Jim White from Plant Protection and Quarantine.

LOUISE HENDERSON: I'm Louise Henderson from the Center for Veterinary Biologics.

JOHN HAMMOND: John Hammond, USDA-ARS.

MICHAEL BRENNAN: Mike Brennan, Center for Biologics, Food and Drug Administration.

NORMAN BAYLOR: Norman Baylor, Food and Drug Administration, CBER, Office of Vaccines.

KATHRYN STEIN: Katie Stein, Division of Monoclonal Antibodies, Office of Therapeutics, CBER.

KEITH WEBBER: I'm Keith Webber with the Division of Monoclonal Antibodies and the Office of Therapeutics of CBER as well.

JULIAN MA: I'm Julian Ma of Guy's Hospital in England.

JOE JILKA: Joe Jilka from ProdiGene.

CAROLE CRAMER: Carole Cramer from CropTech Gene Tech.

MARY DITTO: I'm Mary Ditto from the FDA Center for Food Safety and Applied Nutrition.

BARRY HOLTZ: I'm Barry Holtz from Large Scale Biology.

ALLEN MILLER: I'm Allen Miller from Iowa State University, study replication in genus question.

MICHAEL HANSEN: Michael Hansen, Consumers Union.

CHARLES RUPPRECHT: Charles Rupprecht, National Center for Infectious Diseases, CDC.

DAVID ESPESETH: Okay. We're open for questions. A member of the panel, if you have questions of your colleagues, please help me out.

Yes.

RACHEL TEE: Okay. I'll just go ahead and ask as long as I can. My name is Rachel Tee. I'm a graduate student at Iowa State University, and my question is to both the practitioners and the regulators, and it concerns the use of antibiotic markers in transformation. And what I want to know is for the practitioners, is it a concern when you actually take the transgenic tissue without purifying the protein and feeding it to an animal or a person? And what I want to know from the regulators is that is it an issue for regulation, in your opinion, and what is in place to look at that, if at all it is an issue? Thank you.

DAVID ESPESETH: Okay. Regulatory question concerning antibiotic markers. Louise, you want to start?

LOUISE HENDERSON: For veterinary vaccines -- for all of our veterinary biologics, you should be talking to us if you think that you need to leave an antibiotic marker in your product.

We would recommend to you that you take those out of your final construct. We are concerned about the spread of antibiotic resistance markers in the environment. However, we will deal with this on a case-by-case basis, and it depends upon the need to leave it there and on which antibiotic markers might be used.

Certainly any that are used for therapeutic use in humans or animals should not be used in final product. And our recommended for that has to do with the filing of the information format in which all of the genetically inserted information must be stated, and our risk analysis

and risk analysis with an antibiotic resistance marker present is different from that of a product without an antibiotic resistance marker.

DAVID ESPESETH: CBER?

KATHRYN STEIN: We have seen the use of antibiotic resistance markers and antibiotic selection in fermentation for human biologics.

Penicillin should not be used as an antibiotic. Kanamycin is most typically used and we usually ask the manufacturers to demonstrate that they can remove the antibiotic during your downstream manufacturing. That is certainly the case if it's used in large-scale fermentation.

Occasionally, Kanamycin is used only in the earliest stages of culture, and then it is not an issue. But if it is used in the fermenter, then we ask that validation be done to show it can be removed.

BILL PRICE: From the Center of Veterinary Medicine standpoint, I think also from CFSAN, we have the product in the Kan-r gene is approved as a food additive to be used in foods and feed. So that particular marker is approved as a food additive.

MARY DITTO: There's also guidance available the use of antibiotic resistance markers, and that can be found on our Website if anybody needs it, www.fda.gov. Go to bioengineered foods. You'll get to the CFSAN's Website with all the pertinent guidance documents, and you can find it there.

DAVID ESPESETH: Other questions?

GUY CARDINEAU: Okay. Just to give a response from the industry side instead of regulatory side, it is our common practice to remove all antibiotic resistance markers from constructs.

Anyway, if we're looking at bacterial antibiotic resistance, that might be used in the selection of the original constructs when being assessed in the bacterial systems.

Those are purified away from the expression cassettes that are actually mobilized in the plant. It's particularly important when you're dealing with the monocot transformation systems that are not using Agrobacterium. If you're using a physical delivery such as a particle gun or whiskers or some other mechanism, you then purify away expression cassettes from the residual bacterial. In the case of Agrobacterium delivery, the DNA between the border sequences outside -- I discussed this yesterday -- is what's delivered. So your bacterial antibiotic resistance determinance would be outside of that area.

In relation to the material that would be used for selection of plant cells, in general most folks seem to be using herbicide selection; for instance, PAT selection, FAS 3 transference. Some other genes may be used as well.

But generally speaking, I don't think anybody is using antibiotic selection for plant cells for things that are designed for commercial production.

I do have a question for the regulators in that regard. Is there a difference between the use of antibiotic selection markers that would be driven by plant promotors versus those that would be bacterial with regard to your concerns? And the reason I ask the question is that there's a concern about animals acquiring antibiotic resistance determinance. Certainly the gene in itself without regulatory signals would be an effect of Agrobacterium. So I'm just curious. I want to make sure we understand what the rules are.

LOUISE HENDERSON: I do believe that there are some concerns that we have. It has to do with the ready availability of those parts of genes and certainly of antibiotic resistance genes already in the bacterial populations found in animals.

Certainly there's a number of animals that are exposed to antibiotics on a fairly routine basis in which that is a concern. So we do have concern about antibiotic resistance markers.

However, we do believe that it's very easy for selection to be made not on the basis of bacterial antibiotic resistance markers; as you've said, taking those markers out. Certainly we do not have any problem with using antibiotic resistance markers in the design and the construction of a vector, but it should not appear in the final product, and it should not be part of your final cassette unless you wish to address the environmental issues that will be raised by use of that gene or part of that gene even without the promotor.

DAVID ESPESETH: Other comments on that issue?

NORMAN BAYLOR: Yeah. That would be the same from the biologics side also. The concern is the antibiotic resistance in the environment.

MARY DITTO: When CFSAN developed its policy and looked at the use of antibiotic resistance markers, several things were considered.

One is the expression of the resistance protein in the plant. Many of the constructs, the early constructs that were used in the generation of transgenic plants, were under the control of procaryotic promotors. We do not expect that they would be expressed in the plants.

And for those things that we don't expect to have expressed in the plants, we have evidence that they are not expressed. There are markers of note in Kanamycin that has been assessed as a food additive that were used as selectable -- it has been used as a selectable

marker for plants. The protein -- the APH-2 that confirms the resistance to the antibiotic is digestible. It's expressed at low levels and doesn't represent a safety concern as used.

The question of gene transfer was looked at. What are the possibilities for gene transfer from a plant to a bacterium, and what is the occurrence of antibiotic resistance in natural populations of bacteria and the background, so to speak, that we're looking at?

And those issues were taken into consideration when those first constructs were considered and put into plants, and we had no reason to pose any restrictions except we did advise that certain genes be avoided, especially a note on the side of precaution, not to use genes for clinically -- last-resort antibiotics.

So Vancomycin, those kinds of antibiotics, we say don't use that gene. We would have strong reservations about that. But other antibiotic resistance genes have been used and have been assessed at this point. Do you have anything to add, Bill, from the animal feed side?

BILL PRICE: In trying to look at those genes that had bacterial promotors -- and we brought in a number of experts and consulted with them and looked at -- trying to look at the possibility of the transfer from the plant to the bacteria in the animal.

And I think while it's theoretically possible, it is very, very unlikely based on the experts that we consulted with, which means that the book is still open in a way, and I still think that as far as the agency is concerned, we'd rather not have those there if we can avoid it.

But on the other hand, we hadn't seen the safety factor.

DAVID ESPESETH: On the end.

MICHAEL HANSEN: Yeah. This is Michael Hansen. I have a very different take on this.

I should point out two things. Even though the Food and Drug Administration did indeed license the use of the Kanamycin antibiotic resistance marker gene, there was dissension within the agency as part of the lawsuit that was brought against the Food and Drug Administration.

And 40,000 pages of documents came out during discovery. Among those were -- I don't have them here with me. They're actually back in the hotel. I would have brought them.

It turns out that the head of the what is the Center for Anti-Ineffective Drugs --it was an M.D. -- they came down very strongly against the use of the Kanamycin resistance marker gene and actually have done some calculations for the first product for the tomato that went on.

They calculated background levels of that protein in the environment that you would be ingesting of 2.8 times 10 to the minus 6th micrograms per person per day. With an average diet of tomato solids, that number was micrograms. That's almost a 20 million fold difference.

And it's a four-page memo. I actually have it here, and you should know that there was dissension within the agency on the medical side. They went for it anyway.

Also on the Novartis Bt corn, the selectable marker on that was actually glufosinate resistance, but it encountered resistance in Europe because it also had Ampicillin resistance. That was a carryover from the Agrobacterium transformation.

So I think there was some concern that since that is under a bacterial promotor that there could be some movement, and that's why I know the United Kingdom and four other European countries were adamant against that Bt corn variety because in the U.K. they said Ampicillin is used in animal and human medicine, and even if the risk is a small one, there's no reason why it needs to be here, and they wouldn't permit it.

So there's a difference, I think, internationally, and it appears that there was some debate within the agency. So I think the issue is not a closed one. It's probably going to rear its head again in this country and also internationally.

DAVID ESPESETH: Any other comments on that issue? It appears the working group will need to address that in their guidelines to give the industry some direction as to how to proceed as far as use of antibiotic markers in the selection process.

Yes, sir.

TOD STOLTZ: I hate to beat this issue to death, but my name is Tod Stoltz from Applied Phytologics.

DAVID ESPESETH: Please speak up.

TOD STOLTZ: Sure, sure, sure. It's pretty much universally considered in biotech, I think, these days that the antibiotic marker is going to be out with all the transgenic plants when we do transformations, but the selectable marker for the herbicide is still an issue, and it have some good clear guidance from all the regulatory agencies as to what selectable markers are available to be used and whether or not it's an issue if the gene is there versus the protein.

There's a possibility to use targeted expression in your selectable marker so that the protein is not present in the final product, but the gene still will be there.

It would be good if we knew if that would be a regulatory concern for all of the agencies or if that's not an issue for all the agencies.

DAVID ESPESETH: Could you respond to that question, regulatory officials? Plant geneticists?

LOUISE HENDERSON: I think for animal biologics that issue was still under consideration, and we have not made any decisions. And I'm sure that the whole working group would like to discuss this issue and see if we can come up with a coordinated response to that that we would all be comfortable with. So I guess the jury is out on that one at this point for us, at least.

JOHN HAMMOND: I'm not a regulator, but I mentioned in my talk yesterday that for purposes of eliminating transgenic plants remaining in the field, there's a necessity to have a legally labeled herbicide available that will be able to kill those plants, so that is one consideration to take.

But I have no opinion as to whether there's an advantage or disadvantage to the protein being expressed, just the fact that you should leave yourself the ability to kill those plants with a legally registered herbicide.

WALTER GOLDSTEIN: Hi, Walter Goldstein, Biolex. I just have a question, the information thing.

What is the extent of the presence for antibiotic resistant organisms of the type that already resists as resistance factors that we've been discussing in the human body, in the environment? Is there any information on that?

Because if they're already there, then, you know, it's a different situation than worrying about creating that resistance. Maybe someone could help me on that.

MARY DITTO: At the time the Center for Food Safety had an advisory committee meeting to discuss the use of antibiotic resistant markers, that question came up.

Depending on what you're looking at, the location in the soil, the particular antibiotic -- I mean I'm not going to speak from the animal side, but there is certainly information available. There can be more information.

There's certainly prevalence of many of these products already in the environment. The question becomes in part what Dr. Price addressed. What's the possibility that this gene is going -- that gene in particular will be transferred to soil bacterium, and that will give that bacterium the chance to grow out? Now, I guess you could say you can eat it. Is there any -- and it was discussed was there any evidence of transfer of genes from plants that we eat into the endogenous microflorabof the gut?

Again, it's something that if it occurs, it's not a frequent event, and if it does occur, would it grow out to become of some significance? What's your selection? And at the time for kanamycin, it was thought that the -- having looked at that question fairly carefully that it was not a concern, a safety concern, from our point of view.

LOUISE HENDERSON: I'd like to say that from our point of view, the issue is not the safety to the individual animal that's going to hit the product. Obviously, that needs to be covered during prelicense data submission.

But our concern about antibiotic resistance markers has to do with being responsible stewards of the environment, and we know that using antibiotic resistant markers in live genetically modified organisms is not a responsible use of those markers, and we have not been approving use of most antibiotic resistant markers for live organisms.

The use of plants is, of course, somewhat different, and we haven't really addressed all of those issues, but we have a concern about the spread because animals do have a arge number of antibiotic resistant marker carried in the bacteria that they have in their guts and on their skin, et cetera.

So it's important to us that we limit the spread of those, whether or not it's a danger to that individual animal.

DAVID ESPESETH: Another question.

GREG BOBROWICZ: I'm Greg Bobrowicz. Question for CBER, I think. What's going to be or what's the current thinking, regulatory expectation, on viruses, plant virus, in both characterization of feed stream as well as steps for removing them in downstream processing?

Are we considering that as a consensus that it's very likely that we're concerned about viruses carrying over, and if so, is it an autogenicity issue, a pathogen issue? What's the thinking on that?

KATHRYN STEIN: Others from CBER may like to comment, but I'd like to start. I think that we would not ask for validation studies to show that these viruses could be removed if the process is a robust purification process and we have no concerns about possible infectivity in humans.

However, we would ask, I think, as a matter of course that adventitious virus screening be done on the unpurified bulk product to ensure that there are no viruses that can infect mammalian cells, and we would ask for adventitious virus testing on the usual panel of susceptible cells such as MRC-5 and VERO cells.

Does that answer your question?

GREG BOBROWICZ: Yes. Thank you.

KATHRYN STEIN: Does anybody else want to comment on that?

KEITH WEBBER: I might add just a possibility that if you have a particular – if you have a virus that you produced that has a target cell that that cell line may be added to the list of cell lines that you'll be testing in the adventitious agents testing as well, just in the event that you end up -- we don't know yet whether viruses can be pseudotyped to enter an animal cell by adding a protein to a service which would allow entry into the animal cells. That's something that could be looked at as well.

GREG BOBROWICZ: I'm pretty confident I understand what you just said. Are you saying that if I have TMV in my plant that I should dump TMV on the cells to make sure it doesn't infect? I didn't understand your thoughts.

KEITH WEBBER: You should test your plants so that -- I don't know about TMV, but I was thinking in terms of if you're targeting particularly perhaps for vaccine, if you're targeting a cell line -- if you put a protein on the surface of a virus, will it allow it to enter into a cell. Will it have an effect on that cell?

GREG BOBROWICZ: I understand.

BRANDON PRICE: My name is Brandon Price. I'm from CropTech, but in a previous life, many previous lives, I was involved in biosafety testing.

I think what Dr. Stein is referring to is if you're making biologic products in mammalian cell culture, there's a standard list of susceptibles, what I'll call susceptible cell lines like MRC-5 and VERO and others, and that list is typically three to five cell lines.

And it might depend upon the origin of the product whether it's a human cell that's being used to produce it or a nonhuman cell, but you're looking for -- what you normally do is you mix your unpurified bulk material, you know, with -- or cocultivate with the cell lines, and if there's a transfer of virus, you can use standard cytopathic effect or other types of -- looking for the presence of the viruses, and you can determine whether or not viruses have been in your product.

And I guess my question to Dr. Stein is, are you referring to that kind of test as something you might expect a manufacturer to do?

KATHRYN STEIN: Yes, I am referring to that kind of test. Although we don't expect TMV to infect human cells, we want to ensure that there is no virus present in your product that

can infect human cells, and that's a safety test that's done on all of our biologics for parenteral use.

There may be some differences in the testing for oral use products. In addition, there might be differences depending on the type of product that you're talking about. I think that's what Keith was getting at.

If in some way the virus that you're using as a vector is altered because it's expressing a product intended for human use, it might alter infectivity of that virus.

So I think that we would need to look at each product and determine the appropriate type of testing needed, but we would not ask for the usual types of viral clearance studies that are typically done with retroviruses and other model viruses for cell lines that are known to produce endogenous retroviruses. We've not asked for that kind of testing.

DAVID ESPESETH: Other questions?

Yes.

JULIEN MA: Hello. I have a question for the regulators. Given that none of the companies here who are trying to produce products are likely to be able to contain their marketing within the U.S., what efforts are being made to coordinate your guidelines with other regulatory bodies such as the ones in Europe?

NORMAN BAYLOR: I guess I'll start. Both of the guidelines and regulations that we develop in the agency, we're in close contact with our counterparts in -- we have the ICH. We work closely with the EMEA in Europe. And so we're not doing this process in sort of a vacuum. We're constantly in contact with our counterparts around the world.

LOUISE HENDERSON: For CVB we are so in our infancy in developing our guidelines, we have not yet opened those discussions, but we have had discussions within our agency about bringing those in line with us, bringing those discussions with other governments at the appropriate time.

However, we do need to have some development yet before we're ready to go that far. I'm sorry.

With working with the FDA, we assume that we are going to have a great deal in common, not just within the country but within the world for products made through transgenic plants, whether or not they are made for human or animal use.

KATHRYN STEIN: I can tell you what was done with the points to consider for monoclonal antibodies for human use with the '97 guidance document.

When guidance documents are written, they go through internal discussion within the working group writing it, and then after that the document goes through agency distribution for comment. And in this case as a joint document, it would go through USDA as well as FDA.

When the points to consider for monoclonals was written this 1997, at that stage of review before opening it to public comment, it went to the authorities in Europe, Canada, Japan, and Australia as representative of a worldwide group, and that's something that would likely be done.

So this would be a document at the stage that the working group feels that they have a clear document but before it goes out for public.

And then once the comments are back from all those authorities and from the centers, then it would be made available through the Federal Register.

DAVID ESPESETH: Another question from the audience.

CHRIS WEBSTER: I'm Chris Webster from Pfizer. I'd just like to ask whether there's been any consideration given to the physical security of these recombinant lines?

It occurs to me that you may have tens, hundreds, or even thousands of acres growing these plants, and what's to really prevent strange people coming in and taking them away and growing them somewhere else, which would be an impact on the intellectual property of the company, actually has profound regulatory considerations as well.

We've seen it on the vaccine side where modified live seeds have wandered off and have appeared in other products. I wonder whether the panel has any thoughts on this?

DAVID ESPESETH: Yes.

JOE JILKA: For one comment I'd like to make is I think especially if you grow a few acres of corn in Iowa, the best way to secure it is to grow it just like any other corn. In other words, the anonymity of it just completely hides it.

You know, our TGEV corn grown was up here by Story City right by the interstate, and no one could have ever seen it. To secure it and build a fence around it is essentially to put a sign on it and say, "This is where it's at. Come and take me."

Otherwise there's absolutely no way to tell what it is unless you had some sensitive thing in PCR or something like that.

DAVID ESPESETH: So the more obvious you are about security, the more at risk you're putting your product.

LOUISE HENDERSON: Clearly, anybody who were to take a biologic transgenic plant and grow that without permission would be clearly in violation of a number of laws.

Of course, the problem would be detection, obviously, but when they are in violation of laws, there can be actions taken to stop that kind of action from continuing.

CHRIS WEBSTER: I just wondered whether perhaps as part of the emerging regulatory scenario it might be mandated that there would be some -- what we heard about the white tomatoes, that there might be a mandatory requirement to have some ready marker which would clearly dentify this as, say, at the very least an unusual plant?

MARY DITTO: Well, the expression of the gene inside the plant, the marker, I mean if you're expressing a biologic, that's the marker in the plant. It may not be readily recognizable, but to an expert or if the company knows they're being -- someone is pilfering their product, they would be able to find it fairly quickly based on that.

KATHRYN STEIN: We have some discussions in the working group about markers for edible vaccines, primarily to aid in the delivery of those vaccines but not as a security or identification measure that says, "Come steal me. I'm a recombinant vaccine."

But we have not come to any firm decisions, and I doubt that it would be a requirement. I think it's very unlikely, but we don't have a final decision on that.

DAVID ESPESETH: One of our speakers presented some ways of having color markers for expression in the product, so I think if it was required, there are ways to address it.

KATHRYN STEIN: I think the advantage would be, again, if it were a vaccine, an edible fruit used as a vaccine where it might be desirable to distinguish it from other fruits. But for plants used as source material for biologics, I think it's very unlikely we would ask that they be marked in a visible way.

DAVID ESPESETH: Probably take some other approach on your GMP for assuring proper control of the materials.

JIM WHITE: I'd like to say that I mentioned in my talk that these products are not going to go under the petition process for deregulation, and one advantage for all the companies or universities that are doing this research is that when they're under APHIS authority, if there's any movement from outside that contained facility, even by animals or something like that, we have legal authority to prosecute those people with a $10,000 fine and a five-year in prison, and we can find that.

And of course, for intellectual property rights, I assume the companies would also want to track those people down. The prosecution would be handled by APHIS.

So I think that's a great advantage for all the developers of this technology to stay under APHIS oversight.

JOHN HAMMOND: What I mentioned as uses of some potential visible markers are things that would not necessarily be apparent to one unskilled in the art but would be of more use for identifying volunteer plants as possibly being the transgenic which could then be confirmed by other means such as PCR.

If you have something like LEGULES or glandular hairs that are particular or at least uncommon, then those can be used to identify the plant as being something unusual. And if you find a volunteer plant, you can then check it out.

But I would be hesitant about using something that would mark a plant as being obviously different so that somebody could come in and interfere with it.

DAVID ESPESETH: Any other thoughts from our commercial companies about security or some other thoughts? We think being a tree in the forest is the best place to hide it.

Yes, sir.

MICHAEL BRENNAN: I have another question.. What biologics can we make in plants, or are there certain biologics that we shouldn't make in plants?

For instance, if we wanted to make a childhood vaccine like the ones that we have licensed now, you have to include diphtheria toxin, tetanus toxin, and pertussis toxin.

So the question is -- and maybe I could ask members of the panel or the audience -- could we make these toxins in plants? Now, we could make them as genetically mutated proteins. That may be the best way. But then maybe they wouldn't oligomerize the correct way. So the question is, if we wanted to make a childhood vaccine fully in plants, can we express these toxins in plants and then purify the protein and toxoid them after the fact?

So maybe I'll open that up as a question for anybody that has any thoughts on that.

JOHN HAMMOND: I would say that any food -- any toxins should not be made in food plants.

MICHAEL BRENNAN: Well, that answers that.

JULIEN MA: We have a little bit of experience working with tetanus toxin, and our experience has been that if you're going to toxoid it anyway, you might as well do the mutation as well as you're doing the engineering before you put it into the plant. So that's exactly what we've done.

DAVID ESPESETH: In order to produce a toxoid?

JULIEN MA: An inactive form of toxin, yeah. I mean that's likely to be much more stable anyway.

JENNIFER CONLON: And just a comment on the toxins. The immunogenic issues about toxins, you don't need for it to be active to be immunogenic, so I can't see where you wouldn't want a toxoid in the plant. It would make perfect sense as long as you still had immunogenic regions there.

KATHRYN STEIN: I think there is an interesting issue about the enhancement of immunogenicity by toxoid and agents like formaldehyde. For example, the CRM 197 diptheria toxin is a more immunogenic toxoid even though it's not toxic. So it might still be toxoided in the end.

LOUISE HENDERSON: I think, too, there's been some interesting research showing the advantages to using toxoids or at least parts of toxoid units to enhance immunity development that are really very exciting when you see the great impact it has on the ability to stimulate the desired immune response.

ALLEN MILLER: To change the subject a little bit, already with tobacco mosaic virus, it looks like they're expressing this ALPHA TRYCOZANTHIN, which is a ribosome inactivated protein, so it sounds kind of toxic to me. And I was wondering how that's been considered?

DAVID ESPESETH: Anyone?

ALLEN MILLER: Jim or Barry?

BARRY HOLTZ: ALPHA TRICOZANTHIN? Was produced by our company mostly as a demonstration molecule. We have no interest in commercializing it at all. It hasn't gone in the field.

LARRY GRILL: Well, it's kind of an irrelevant issue. Almost all plants have the same capsule inhibitory protein, and wheat germs has probably one of the highest concentrations around. They're easily inactivated in the stomach, and again, it's ubiquitous. That's not an issue. We aren't concerned.

BARRY HOLTZ: There was an original interest in anti-AIDS indication on that molecule.

KATHRYN STEIN: Our approach has been to sponsors to ask them with endogenous toxins, plant toxins, and make sure that they would remove them during their purification process for purified biologics.

GUY CARDINEAU: I guess one thing that I'll add is that when we're talking about subjugated vaccines, we're talking about expressing these things in plants, and it's not necessary to express the entire antigen. You can do epitopes also.

Particularly, we look at some of the viral delivery systems. Access Genetics was mentioned here at some point during the meeting, had something called CRY? virus particles, and they actually would express an epitope of less than 30 amino acids on the surface of the virus particle, and they could get fairly reasonable antibody response by doing that.

So there's a whole range of ways, I think, to address this without actually having the entire toxin expressed in the plants.

DAVID ESPESETH: Yes, at the end.

CHARLES RUPPRECHT: I was wondering what's known about bona fide plant virus transient progression, persistence, clearance, shed when consumed by invertebrates, simple stomach ruminants, et cetera? Is it inactivated in the gastric milieu? Is it fermented? Does it come out in feces, secretions, et cetera? Is there any clearance transient studies that have been done with any animals with bona fide plant viruses?

JOHN HAMMOND: I don't know of any studies relating to clearance in that sense, but that are some plant viruses that have significant environmental persistence in water, in rivers. There are a number of plant viruses that can be transmitted and can be isolated from lake and river water.

The question is, how do they get there? In part they may be leached from plant roots or from decaying plant material. I don't think that point is yet clear. I have no knowledge of any studies of biological clearance from the gut.

CHARLES RUPPRECHT: Do you think, would they persist in mammalian gastric milieu? I mean are most common plant viruses inactivated by he kinds of pHs and enzymes that you find in --

JOHN HAMMOND: Most are fairly readily inactivated in acid pH or, alternatively, high alkaline pH. They just don't have the physical stability.

LOUISE HENDERSON: I think that issue is one that is likely to be addressed during the risk analysis process and may indeed, depending on the virus, require some sort of testing to demonstrate that.

DAVID ESPESETH: Any other comments on that or other areas, either production or standards for products, issues that need to be addressed? Yes.

BIPIN DALMIA: Bipin Dalmia from Novartis. Along the lines of the physical security question, is any consideration given to natural disasters such as floods and tornadoes?

DAVID ESPESETH: Natural disasters. How about that? Security in natural disasters or --

JIM WHITE: Well, this was actually in Science, I guess, years ago in the early '90s. There was a flood here in Iowa, and presumably some transgenic plant plots were destroyed, but you know, I think all the evidence was that they were under several feet of mud, which probably is a good way of decomposing them. And you know, that's, you know, a pretty rare event but not zero. And I think flooding, you know, I guess I've been through near a tornado. I think those plants are pretty well destroyed and stuff during that kind of event. But that's something the agency will have to think about.

BARRY HOLTZ: I can speak fairly firsthand about tornadoes. We just had a heck of a big one in Owensboro, Kentucky, and just to add a little levity to the situation, we were just damn glad to be around after it was over, to tell you the truth, and really weren't concerned about whether we lost a tobacco plant or not because it landed in our front yard.

But we do plant fields in diverse areas of the county. We don't concentrate our fields, and that's mainly a risk management on our part in case there is a hail event or wind event or something like that that would kill the plants. We move around.

JIM WHITE: I'd like to make another comment, is this is what John Hammond talked about is the crop biology that you chose, you know, corn, even if there were ears of corn, you know, kind of stuff and they were blown, you know, to the next county kind of stuff, you know, corn doesn't persist. You know, it has to have human intervention.

So even in that unlikely rare event, the biology suggests that there's no evidence that that corn plant would persist in any way. Again, if they're not visibly tagged, there would be no reason for anybody to save that ear of corn.

Of course, if there was damage by a tornado, I would think that you wouldn't really care about that. Other issues at that time.

So I think the biology of the crops also that are chosen would favor the natural disaster would not be a significant impact.

KATHRYN STEIN: I have a question for Dr. Henderson. We have investigational new drug exemptions, INDs, for experimental products, and we would regulate the interstate movement of products under IND. You don't have INDs, per se. How do you regulate the movement of experimental products, and at what stage would you get involved?

LOUISE HENDERSON: It becomes a very interesting question as to what stage we would get involved with this kind of product. We regulate that under our 9 CFR 103.3, which has to do with shipping of an experimental biologic, which we do have authority to regulate that process, and that's what I talked about, our need to give you permission to ship or move that product.

And we are having discussions within our agency as to the appropriate time for us to become involved with these products, but certainly, we do have the authority for any plant that expresses any biologic vaccine that is intended for use in animals to be under our regulation. And we should be giving permission prior to shipment or movement of those products.

We would much rather have you contact us and ask us about the need for permission than we would to have you assume that you do not need to get permission from us. We would be happy to work with you to assure that the safety standards are met and that all of our regulations are met.

Many of our regulations do not cover what happened within your own facilities, but once it goes off of your property, our regulations go into effect.

KATHRYN STEIN: Let me just give you a hypothetical scenario. If somebody is making an animal vaccine in a plant that they intend to use as an animal vaccine but it's in the very early stages of development and they ship it across the street to a laboratory for preclinical testing, does that come under your regulations?

LOUISE HENDERSON: Yes, it does.

KATHRYN STEIN: Even though it's not going into animals?

LOUISE HENDERSON: Yes, it does.

KATHRYN STEIN: It does. Okay.

LOUISE HENDERSON: Shipment of experimental biologics is covered under 9 CFR 103.3.

DAVID ESPESETH: There might be a difference as whether it was containment to containment in terms of the amount of time it would take to get approval rather than containment to release, but in either case a permit would be required.

LOUISE HENDERSON: It's unlikely if it's containment to containment that there would be much of a delay in approval. If it's containment to release, obviously, we need to do some risk assessment at some level.

GUY CARDINEAU: Louise, can I follow up on that? I just want to make sure I understand this. This actually comes back at some point. If we had, say, leaf material that was frozen in liquid nitrogen, we would not be able to transmit that across the street without a permit from you folks? I don't have a problem with that. I just want to make sure I understand.

LOUISE HENDERSON: Yes, that is correct. We do have authority over that movement.

BARRY HOLTZ: We have been through this drill many times, and USDA has been very attentive about getting people, inspectors, to these sites and then facilitating these transfers. It's done very smoothly and really don't have any problem.

JOE JILKA: I have an additional follow-up question for Dr. Henderson on that. Would you -- and maybe you haven't quite decided this yet. Would that also apply to material that's, say, in back-cross program, in the breeding program?

LOUISE HENDERSON: Our authority does go to that material. We will be making some determination as to what level of control we will want to be involved in with all of these types of products.

But you would be better off asking us ahead of time before you move any material because it clearly is a biologic once it is expressing an animal vaccine marker.

KATHRYN STEIN: I just wanted to point out that on the handout that has Bill Price's diagram of who regulates what, there's a list of contacts for all the agencies, so please make sure you take one.

LOUISE HENDERSON: Actually, I did realize earlier that all the copies of that were gone from up at the desk. I'm sure that there will be more later, or you can leave your name, and we will get one to you if you did not get one.

DAVID ESPESETH: This material will be posted on the Internet as well?

LOUISE HENDERSON: Yes. All of the material from this whole two-day seminar and public meeting will be posted on the FDA's Website. We will link to that from our

Website. We won't repost everything, but all of this information will be available once the court reporter has had time to go through all the scientific terms that she may or may not know. And so we're hoping in six weeks that that information is available at the FDA Website. We've got that in many of your spots in your handout as a list of the FDA and address for the FDA Website. And you could always go to our Website and find that link once that's available also.

DAVID ESPESETH: As you develop your guidelines, will those drafts of that be made available, or will you only issue a final draft for comment?

KATHRYN STEIN: I think it's our intention to have a complete draft that's gone through agency clearance and then make that available for comment. It will be a draft document at that point as it's issued. It will not be finalized until it's been out for public comment.

DAVID ESPESETH: Any other questions concerning any of the papers? Yes.

DAVID STARLING: I'm Dr. David Starling with the Center for Veterinary Biologics. Just a basic question for the panel, perhaps more along the line of developing regulations for this new technology. I'm wondering what thought has been given to comparing some of the gene sequences that we're moving around to some of the normal data bases or some of the future gene data bases, just to rule out the possibility that maybe we have a dually active gene sequence or something else.

DAVID ESPESETH: Anyone on the panel want to expound on that?

LOUISE HENDERSON: We have a number of scientists within our agency that review all of the SIFs. I'm sure that's something that we consider and perhaps should consider more carefully, but it is an issue that as the gene banks grow, we can certainly look at that more fully.

At this time many of our SIFs have done gene bank searches. I'm not convinced that I know whether or not all of them have done that at this point, but then a SIF as presented to us is not considered final until we have approved it. So it's certainly part -- that can be part of the risk analysis procedure.

ALLEN MILLER: I was just going to say I think -- I think it might be a good way – I think most scientists probably take their favorite sequences and regularly screen them on data bases, but it might be a way, you know, to answer this question that has come up, for example, about plant viruses infecting humans.

I was thinking about it last night and thought one way to maybe find out how much exchange there's been is take entire plant virus sequence data base and compare it against the entire human EST? library data base and do these big comparisons and look for homologies.

And they may indicate that there has been a plant viral gene transfer, or they may indicate that -- I mean there could be infection, or it could just indicate that there might be blocks of homology that don't have anything to do with that. It would take further investigation, but it might be a way to explore those possibilities.

LOUISE HENDERSON: Yes. I think hat's very true. One thing that happens in our agency for all of our recombinant organisms is that our laboratory characterizes all genetically modified organisms at the molecular level, and a gene search is part of that analysis.

JOHN HAMMOND: I think one would also have to be fairly careful about that because a lot of times when people prepare seeding from plants -- and I'm thinking especially from tomato -- that an awful lot of the clones that have been recovered to tomatoes antivirus.

So the fact that you find something reported somewhere is not necessarily evidence of insertion there but potential contamination.

ALLEN MILLER: Yeah. I think what I thought you were going to say was you could get human DNA in a plant library. I mean that could be the bigger problem. Humans are doing the cloning and I know that's happened too. I mean I've seen people make CDD? libraries from GNA PICKS? that were dropped on the floor so -- it's true. I'm not going to name names but -- it wasn't my lab. No.

But you have to investigate further and see what's going on. And personally, I'll just go on record and say I think there's virtually zero chance of a plant virus infecting human. There. Prove me wrong.

BILL PRICE: Bill Price. I'm just wondering, our friend here from the integrity for seeds, if there's any move to map, like, the corn plant or soybean plant as far as what different genes would be available in all aspects. I know that there's a movement on the part with OECD to define the primary nutrients and anti-nutrients and so forth in various plants, and we at FDA are working on a project with the soybean, but it isn't anything to do with the gene mapping.

But on our Website for those plants that we approve for food and feed -- or not approve. I shouldn't say that -- that we've looked at for food and feed use, we have identified those genes on our Website, but I'm not sure as we go further into this how one is going to know what all the genes that are available for the various plants. Was there any discussion on that?

JOHN HAMMOND: There are genome efforts on, I think, all of the major crop plants, certainly wheat, corn, soybean. There's expressed sequence tag libraries. I think all of that information should be available over the next few years.

There are already extensive gene maps for potato, tomato, pepper, I think several other Solenaceous crops. Those libraries will be filled out over the next few years, and complete sequences should be available for some of the crops over the next few years.

BILL PRICE: Do you think they'd incorporate these special genes that are now being inserted?

JOHN HAMMOND: Those libraries, I don't think, take account of any transgenic material that is just the basic genome, but that would allow examination of into which genes transgenes have been inserted.

But other than that, I don't think that transgenes would be included in those data bases.

MARY DITTO: Well, once you have a genomic location, once these genomes or sequences -- assuming they're publicly available, then when you insert your genes, you can look at the board of sequences and know precisely where you inserted your gene.

That may alleviate some of the concerns around unexpected effects or hooking up open reading frames and producing proteins you don't expect. You'll have that information fairly readily available, I would think, in short time.

JOHN HAMMOND: Yes. I would agree with that totally. One of the things that has been of some concern to people producing transgenics is that although it's common practice to produce hundreds -- certainly tens and frequently hundreds of lines, in order to select a line that has the parenteral characteristics without any SOMOCLONAL? variation or other unwanted obvious phenotypic effects, there is always the possibility that you will end up with an insertion into a region of the genome that is only required under specific conditions.

For example, if you have an insertion into the ADH gene, which is only induced under the stress conditions, you might not ever know about that until your plant was stressed in the field, and then they might not be able to respond to the stress.

So certainly it would be beneficial to be able to do that kind of search and find out where your desired lines have the insert.

CAROLE CRAMER: I would respond that that isn't necessarily a regulatory issue but a manufacturing issue, which is that by the time we're taking anything close to the market, we've taken it out in enough acreage and enough generations that there's going to be no issue of those sorts of things, that really when it comes down to brass tacks, it's a very pragmatic sort of thing that we're selecting the lines that perform in the long term.

And when you go to a commercialization sort of concept, you really think differently than -- I mean those things would have been tested, and it's a matter that if the line doesn't

perform for us in the long term, it's not going to be put before the FDA as something that we want to move to a product.

MICHAEL BRENNAN: I thought I'd give Dr. Mason a chance to participate. From your evidence so far, do you think it's going to be feasible to use individual fruits and vegetables for immunization, or do you think you're going to have to batch?

HUGH MASON: I think it's really going to be essential to do a batch preparation and especially if you want to deliver it to a very large population. I think it could be feasible to deliver it to very small populations, but again, it's difficult to quantify the antigen levels in a very precise way.

So I would like to go on record as saying I think it will be essential for us to prepare batch preparations of some kind, and those could be of many different kinds, either perhaps lyophilized preparation ground up to a powder. It could be constituted into a pill of some kind.

There are obviously many different preparations that could be contemplated, but I certainly think that it will be necessary to do that in order to accurately deliver a very precisely known dose.

NORMAN BAYLOR: Have you given any thought to how you would define your lots?

HUGH MASON: A little bit. I think -- actually, in our research we have not done any field studies, so all of our material is grown in a greenhouse, and we would define a lot as basically a cloned batch of potatoes or a homozygous line of tomato seed that were sprouted, germinated, and grown in a single greenhouse or perhaps in a series of greenhouses in the same facility and harvested within a few weeks of each other and then bulked together and prepared at the same time and in the same facility.

That's just my suggestion. Again, we haven't really thought very much about it, but obviously, we do need to think a lot more. Especially if we're contemplating the use of growing material in the field, would we be able to batch together material grown in separate fields in different locales if they were processed all together in the same plant? I don't know. I think we would have to examine how variable the content of those different fields were.

DAVID ESPESETH: Lots of practical issues to be resolved yet. But not impossible.

MICHAEL HANSEN: Yeah. This is another question for Dr. Mason and then potentially one for the regulators. Do you think any of the human biologics that are in plants will be presented as individual fruits or vegetables, or are they all going to be processed further? Because if they are going to be done as individual food items, then I have a question for the regulators but I guess –

HUGH MASON: No. I think at first many years ago, eight or nine years ago, we had a naive idea that that might be possible, but it very quickly became apparent that there could be great regulatory issues that would have to be considered strongly if that were the case.

So certainly now we believe that processing will be completely necessary and that we don't really contemplate delivering individual fruits

I just want to -- a question for Louise. I guess I'm looking for a loophole about the movement of recombinant plant material. Can we distinguish perhaps the use of recombinant material expressing a protein from that under development in a company?

Let's say we're just doing basic research on immune responses to a protein, and we don't call it a vaccine or vaccine in development. Can we take that material across the street to feed it to some mice at another facility if it's not really a product under development, or do we still need your permission?

I know your regulations are written very broadly, so it's probably easy to say that it does fall under the regulations but –

LOUISE HENDERSON: It does fall under the regulations. It really does. But you would have to distinguish that it was an actual vaccine antigen.

HUGH MASON: Anything can be an antigen.

LOUISE HENDERSON: For an animal disease. For an animal disease. If you've got a marker that you're using for some research and it's not a marker for an animal disease, then we would not be concerned about that.

Also, the best bet is to keep it on your own facilities. Now, if you're within a university, that's all a university's facilities.

HUGH MASON: Okay. Excellent.

LOUISE HENDERSON: You should, however, realize that all of this work should be done under your IBC approval.

KATHRYN STEIN: I want to come back to the question of individual fruits. From what I understand from Dr. Mason, currently it doesn't look feasible to use individual fruits, but with this group of plan geneticists here, I'd like to ask whether anybody envisions a better expression system that would allow for a more uniform expression in individual fruits because this is something that we have considered in the working group that we would need to address in the document if individual fruits were going to be used as vaccines.

If it looks like it's not going to be feasible in the foreseeable future, then it might be something we wouldn't waste a lot of time on in our guidance document but put something in there that it would need to be addressed if it occurs.

But I'd like to ask all the plant geneticists to comment on the expression systems hat would ensure a more uniform expression.

JOE JILKA: We're moving a number of human antigens into corn. We feel that, you know, it has the better overall expression, more uniform expression, you know. It can be grown in distinct lots that -- I don't know. That's coming down the road.

KATHRYN STEIN: But do you envision, then, ears of corn would have uniform expression from, you know, within a plant and from plant to plant?

JOE JILKA: Probably, but then that would be -- it would not be given as individual ears or individual -- as a milled product.

KATHRYN STEIN: As a milled. Yeah. That's processing so that's a batch. But I guess what I'm looking for is evidence that there might be good expression systems that would ensure more uniform expression, say, in a tomato plant. All tomatoes or all bananas or all apples or whatever would have good expression that we would be able to give individual fruits.

JOHN HAMMOND: I think part of the answer to that has to wait for evaluation of the range of quantity of the antigen which is necessary to yield the desired response, and that can only be determined through experimentation because there are issues of tolerance and of the effective dose.

And if the level of expression falls within the effective dose, then it could ultimately be possible, but that is going to have to be determined with each antigen and each expression system. But ultimately, it could be possible.

But initially, I would think that purification, puree, and standardization of dose is going to be critical. It's possible that later on when more is known about the systems that you could be right.

BARRY HOLTZ: Let me just comment, too, from a producing biologics standpoint and manufacturing standpoint. Again, from a practical standpoint, we have to have expression systems that are fairly consistent. Otherwise, we can't validate the process.

And to do downstreaming processing and have validated processes, if you have a wildly divergent expression, many times you can't qualify the batch. So I think it's going to be a self-regulating event when it comes to purifying biology.

CAROLE CRAMER: I would say also from the point of view of just plain old plant biology, if you look at the physiology of a seed, a seed is made to provide sufficient protein to allow a plant to go through germination, but if you look at accumulation in a fruit -- for example, a tomato fruit is a very good example where you have a huge variety of amounts of protein and the quality of the protein that accumulates during fruit development.

And if you have fruit being delivered and some is very ripe and some is less ripe, then the potential quality and level of the antigen will vary. So my gut feeling is that it's not going to be a sort of situation, especially in fairly short-lived fruits like tomato, that you would actually go out and be able to assure that it was not just that the antigen was there, but the particular time at which it was fed was consistent.

So it seems to me -- I mean obviously, we're in tobacco. Nobody is going to be eating tobacco. But if I were thinking about it, I would essentially -- if I wanted to feed something, I would go for a seed-based delivery because of the consistency requiring a certain amount of stable protein to allow the plant to undergo germination.

But there certainly are issues with just developmental physiology that would affect your ability to ensure that that reliable dose was there.

But from the point of view of just looking at promotors -- in fact, promotors in plants are quite reproducible, so it's going to be a matter not of whether you can get reproducible expression. It's how that food is then handled that would bring the issues of variability.

KATHRYN STEIN: Thank you. One thing that was occurring to me when John Hammond was speaking was that it might be possible to develop dosing by adding a conventionally produced protein to a fruit, a fruit puree.

For example, you could take tetanus toxoid in different doses and add it to tomato juice and give it orally and then look at the immune response.

And you know, one would expect that the toxoid itself, the protein antigen, would be the same or quite similar through both systems, and you might get a better handle on dosing so that at least you know what to aim for.

And it might be a more expedient way to try to get a dosing than growing fruits and looking at your batches that fruit is grown.

YASMIN THANAVALA: May I comment on that? May I comment on that? We've done an experiment, not for the kind of reason you explained, but we have done it with Hepatitis B using purified recombinant antigen that is delivered as a soluble antigen along with an

adjuvant orally or along with transgenic potatoes from BTI. And the soluble oral antigen, even at a substantially broader dose range, just doesn't do the same kinds of things.

So I don't think, based on our experience with hepatitis, that you're going to be able to figure out doses by giving soluble antigen, spiking it in a drink or a food or a paste or something like that, because I think soluble antigen is handled by the immune system in the gut in a very, very different way. At least hepatitis was.

DAVID ESPESETH: Yeah. I think there was some of that indicated in the TGE work where corn itself delivered the antigen better than if you'd just given the antigen directly.

I don't want to interrupt this great discussion, but we were scheduled for a break at 2:45, and I would leave it up to the audience and the panel.

Do we want to take a break and come back, or do we want to continue until the questions are exhausted? What's the general feeling?

KATHRYN STEIN: Well, there was one question pending, but we should at least get that one. I'm prepared to sit here for a few more minutes to get all the questions out, and then we could take our break as a final -- What do other people think?

BRAD MURPHY: I'll be quick.

DAVID ESPESETH: Okay. Let's proceed.

BRAD MURPHY: Brad Murphy, University of Arkansas. And I want to go back to Louise Henderson specifically with Hugh Mason's question in mind.

And one of your answers about transported materials was really worded with regards to animal vaccines. What about other biologics, therapeutics that are not vaccines at all? Still your permission required? I'm specifically thinking where we have to transport a plant-produced purified therapeutic from one university campus to another within a state.

LOUISE HENDERSON: The definition of a biologic is important here. If it falls under our definition of a biologic –

BRAD MURPHY: Antibody?

LOUISE HENDERSON: Pardon?

BRAD MURPHY: Antibody ultimately for use in humans but at this point –

LOUISE HENDERSON: An antibody for use in humans is not a veterinary biologic.

BRAD MURPHY: Right. So that's whose jurisdiction?

MARY DITTO: CBER. That's a biologic.

DAVID ESPESETH: What's CBER's rule on that?

KATHRYN STEIN: We would not regulate that product until it comes in under IND.

BRAD MURPHY: Great.

CAROL BELZER: I'm Carol Belzer from the Center for Veterinary Biologics, and in all of the discussion on edible vaccines, I'm curious, is there a general mention about allergy-related responses in the delivery with some of these plant antigens? Have you had any incidence of that?

DAVID ESPESETH: Did everybody hear that?

LOUISE HENDERSON: I think we have had discussion about that, and certainly the IGE responses, we talked about our concern.

NORMAN BAYLOR: And I'd just add the same here with CBER. I mean it's a concern, but I mean we do have the sort of staffing and infrastructure to look into these issues. This would be something that we look at.

We do have an allergenic group, and for any product, regardless of whether it's, you know, planted-derived, we would look into it so –

YASMIN THANAVALA: So as part of the clinical trial we recently did on hepatitis, it was a general increase in total IGE or something was a parameter that was evaluated before people went into the crowd and at every time point that they came back for an examination.

We didn't look for potato-specific IGE antibodies, though, and there were no alterations that we found.

DAVID ESPESETH: Other questions relating to safety or standards or clarifications?

HUGH MASON: I have one question about is there a regulation for delivery of plasmid DNA from bacteria at mucosal sites or systemically delivered plasmid DNA which could potentially be contemplated as a use of a vaccine expression system in combination with a recombinant protein from plant or any other source or perhaps as an expression system for an adjuvant?

LOUISE HENDERSON: So you're talking about a nucleic acid vaccine for animal disease?

HUGH MASON: Yes.

LOUISE HENDERSON: That falls under our regulations also. We do have some guidance for DNA or nucleic acid mediated vaccines. Again, if you take it off your university property and it's designed for the treatment of animal disease, that would fall under our regulations.

HUGH MASON: Okay. But my question really was regarding the use of bacterial DNA or antibiotic resistance markers that would necessarily be required for –

LOUISE HENDERSON: For plasmid use?

HUGH MANSON: -- propagation and preparation of the DNA.

LOUISE HENDERSON: Yes. We have actually allowed the use of kanamycin and neomycin resistance markers for nucleic acidm vaccines when it is necessary. Obviously, they must be under the prokaryotic promotors, and eukaryotic promotor then would not allow expression in the animal.

HUGH MASON: Are there similar regulations for human use?

MICHAEL BRENNAN: Yes . I mentioned this morningthis issue is discussed in our points to consider document on recombinant DNA guidelines, which is on the Website (which came about with this initiation of DNA vaccine drugs). So in that document those issues have been looked and at least, thought about.

KATHRYN STEIN: The standard for DNA in biologics used to be 10 picograms per dose or WHO published in '97 an increase in that level up to the nanogram level. I believe it's 10 nanograms. It was a thousand-fold increase in the level. And that, you can find that from the WHO Website. It was May '97.

HUGH MASON: I was under the impression that microgram amounts were delivered for DNA vaccine. Is that not correct?

KATHRYN STEIN: You're talking about the product or contaminants of biologics?

HUGH MASON: The product.

NORMAN BAYLOR: That's correct and oftentimes more than that. I mean those are WHO guidelines. So I mean it depends. I mean it's our own additive case by case. I mean

some of the, for instance -- some of the oral vaccines, oral polio vaccine, it's probably more. I'm sure there's more in the WHO standards.

So we use that sort of as a guide, but depending on product there may be some flexibility there. But there's no codified regulation in the CFR that specifies that amount.

DAVID ESPESETH: Yes.

MARK WELTER: Yes. Mark Welter from Oragen Technologies. And when I feed my transgenic pigs transgenic corn, who's going to regulate the pig plasma for nutrition? And that's just a little levity.

My real point is, you know, this is how fast our world is changing. And the vaccines that we're seeing used in the field are really going to change how products are used, edible vaccines for humans. Has the panel considered how these products will be administered?

You know, Hugh's work, 0, 7, 21 days, that's a lot of trips to the pediatrician. Is this going to be a product that's prescribed and given at home? Has the panel considered that for biological vaccines?

NORMAN BAYLOR: I'll just briefly say that as it stands now, biological vaccines are prescribed like prescription drugs, and those prescription drugs should be delivered by a health-care provider. Whether that changes downstream, I think that would probably require some congressional input.

KEITH WEBBER: I have a question, I suppose for the industry, that in talking about contracting out the growth of, for example, corn that produces a biologic, in some instances the products, if it's a growth factor like EPO – EPO or something like that, there might be safety issues to the farmer himself who inhaled the dust perhaps during the processing.

Do you foresee that when you contract out with the farmer that they would be apprised of what the product is that would be in the corn, or would that be a problem?

BARRY HOLTZ: No, it's not a problem at all, at least in our opinion. We want the farmers to know exactly what's going on, and it's part of what we call farmer training. It's just like GMP training. We expect them to work within specifications so we get a good deliverable. But any perceived risk, we would tell them about immediately. That's full disclosure to the farmers as far as I'm concerned. I can't think of a reason why we would do it any other way.

We might ask them to have a confidentiality agreement about, you know, disclosing business information, but certainly if there's any perceived danger in handling issues, we have to make that very clear. It's just being responsible.

KENT CROON: Kent Croon with Monsanto. As a regulatory affairs person with products, I would echo what Barry is saying. In fact, we discussed this internally, and it was during Borden and working with the farmers or contractors but also greenhouse staff, especially people who were grinding the product.

Once you actually went to analysis, especially with these products, it was as much a concern to make sure they knew there was a danger in grain deaths and dust and so on and so forth many times really, the more dangerous factors than, say, monoclonal antibody themselves. So it was a learning experience when we did that exercise and I think very useful.

JULIEN MA: Can I just come back to the point about administration of these vaccines? I mean this is an issue that's used a lot because as you may remember, our vaccination regime is actually quite arduous.

We feel very strongly that you have to go through the health-care professionals for these vaccine deliveries, and I think it's another very important point why they should be, and that is that you have to gain the confidence of the consumer and the medical profession on this issue.

We've made such a mess of GM foods that if you come up with even any talk about delivery of oral vaccines over-the-counter, I think, then you'll really muddy the water for everyone in this field. It's likely the public will just perceive this as another whack technology idea.

I think we really have to be very straight about this and go through the medical profession as we always have with vaccines.

KATHRYN STEIN: I think one needs to make a distinction between prescription drugs and over-the-counter. We have quite a few prescription drugs that are administered at home such as insulin, growth hormone, interferon, and other products. So I don't think there's any question that these will remain prescription drugs, but there might be a point where some of them might be self-administered at home, and I think that's certainly well in the future. But I think they would always remain prescription drugs, and we would not be over-the-counter.

DAVID ESPESETH: Good point. Another question?

BUTCH MERCER: Butch Mercer from Dow AgroSciences. In response to CBER's response on the medical profession in vaccines for humans, I perfectly agree.

In the case of animal health, though, I don't agree. At this point in time, vaccines or the use of vaccines are left to the discretion of the veterinarian. He may or may not refer that producer by that vaccine from a distributor veterinarian. Distributor can buy it over-the-counter if he wants, or he himself – he or she may elect to administer that product to the particular animal to the herd or whatever, and they are not prescription drugs in the case of animal health.

And to change that in the future, even in light of this unique delivery system, will have significant implications in the industry.

KATHRYN STEIN: I was talking human biologics.

BUTCH MERCER: Yeah. I wanted to clarify because they are such different general markets, distribution methods.

DAVID ESPESETH: Definite difference between USDA and CBER in regards to marketing of these products.

JENNIFER CONLON: Jennifer Conlon from Merial. Dr. Henderson, I wanted to ask you a little bit about duration because some of the work we've seen has been very short duration, and right now in our industry, as you know, a lot of our -- we have grandfathered in durations in a lot of our antigens, and you made a comment which I just wanted a little bit of clarification on that new antigens would require duration. But those are new antigens that aren't currently licensed. You don't just mean new antigens in plants?

LOUISE HENDERSON: That's correct. As far as what duration of immunity will be required data prior to licensure, I can't tell you that at the moment. Those decisions haven't been made.

JENNIFER CONLON: And is there a possibility that what we see with our parenteral vaccines will not be -- that same regulation won't be given to oral vaccines or transgenic plants?

Because right now, you know, a licensed product that's already licensed and don't necessarily have to prove duration. However, you might envision that this delivery system is different, that we can't just assume duration is the same as our parenteral products.

LOUISE HENDERSON: I would be hesitant to really tell you what I think is likely to happen. I don't know. But I do know that duration of immunity is a concern for these products, and I can't imagine that anybody would want to market a product unless they knew what the duration of immunity was.

Certainly it will be a question that we ask for these types of products for oral event plant-based, just as we would for any other vaccine in which we have concerns about how long that duration of immunity might be.

DAVID ESPESETH: Have we exhausted the questions or just exhausted the audience and the panel?

In either case I think this has been an excellent exchange and an opportunity for everyone to clarify and get some answers, and I hope that this communication doesn't stop here and that you will continue to contact the Website as the information comes up there. And if you have written comments, you can certainly submit those comments.

And Dr. Stein, you had a comment.

KATHRYN STEIN: I just want to thank all of the speakers for excellent presentations, and I'd like to thank IICAB for hosting this meeting. It's been a wonderful venue for a meeting, and we hope you'll have us back again. Thank you all.

LOUISE HENDERSON: I would really like to thank all of those who traveled to Iowa to see our beautiful state. We appreciate the fact that we're not a place that you would normally come to vacation, but we've really learned from you, and we hope you've learned from us, so thanks to everybody who's come. I think this has been a very exciting meeting for all of us.

(Meeting concluded at 3:15 p.m.)