And this is a litany of the types of tests that will apply: Spleen and thymus weights, cellularity, body weights. Ex vivo types of tests include the splenic natural killer cell activity; assays of splenic lymphoprolipherate responses to mitogens; in the rat, salmonella type for murine. Antigen is an LPS, like in the mouse, but it doesn't respond as well as the mouse does to LPS-induced responses.

The mixed leukocyte reaction: Here we have a problem again with the rat versus the mouse, in that the spleen cell for some reason has what we call "suppressor type cells" that don't give rise to a very good or robust mixed leukocyte reaction. And so we use lymph node cells in the rat model for that particular assay.

We use flow cytometric analysis. Depending on what in vivo and/or ex vivo type test that we do, we'll look at spleen, thymus, and/or lymph nodes.

We look at cytokine profiles, to try to see if there are any changes in the profiles. We've used the ribonuclease protection assay, which is one where you have several different cytokines that are expressed or can be identified on gels. And we purchase those for the rats. Some of that is strictly TH1, versus TH2 type cytokine profiles.

In vivo tests, which really are turning out to be the most sensitive tests to determine if a chemical is a developmental immunotoxicant--and actually, an immunotoxicant per se: The primary and secondary antibody

response to sheep erythrocytes. You can do that with a platforming cell assay or the ELISA assay. And we've also used KLH.

Delayed-type hypersensitivity response: We've used bovine serum albumin, and KLH, using the foot pad swelling test. And you can also use those animals to measure immunoglobulin responses to that antigen.

We've used the contact hypersensitivity response to DNFB, dinitrofluorobenzene [ph]; looked at penis swelling, ear swelling tests in the rat. And have also used host-resistance models; one including the T.spiralis infectivity model.

So let me just give you--Okay, this is a time line for immune responses to sheep red blood cells, that was published in 1985 by Kimura et al, demonstrating when you could really start to pick up immune function in these animals as measured by the platforming cell assay, with sheep red blood cells as an antigen.

And as you can see, you can get demonstrable effects and responses here at as early as 20 days postnatal. The peak response occurs at postnatal day five--45 here.

This also is the same type of pattern that you see with the T-independent antigens, T-independent-1 and T-independent-2 type antigens, the TNF, LPS types.

So you can measure in the rat at about weaning an immune response to these different types of antigens. If you go

down any earlier than that, you're going to have a lot of trouble picking up anything.

These are the chemicals that we've looked at: di-N-octyltin dichloride, and tributyltin oxide. Di-N-octyltin is used as a stabilizer in the production of polyvinylchloride materials. Tributyltin oxide is a mulluscocide and a fungicide, and is used in a lot of paints and especially as an anti-foulant on ships and boats.

Good old TCDD, one of the most studied of all immunotoxicants.

Methoxychlor, which is a pesticide--one of only four organochlorine pesticides that is allowable in the United States, based on EPA's, basically, elimination of many organochlorine type pesticides.

And then, heptachlor, which is another organochlorine, which has been banned for about 25 years now.

So we have looked at these five different chemicals, and tried to determine: Could we find an effect on the development of the immune system? If we find an effect in the immune system, is it a dose-related response that we see when we look at the immune functional end points?

We also are interested in knowing if this exposure during the development of the immune system is more severe than if one were to do the same dosing regimen in an adult animal,

to determine if there is a difference in the sensitivity there.

Another consideration here is the pharmacodynamics, particularly metabolism of the chemical and its distribution. And I'll give you examples of that as we go through these slides.

The first group of studies we did were organotins. Basically, what we did, originally we looked at the prenatal exposure, and found that there were no effects whatsoever on the immune system of these rats.

We then decided to go and look at the newborn animal, starting on gestation day three, through 24; dosing those animals over a period of time, for a total of ten doses, with either the di-N-octyltin dichloride or TBTO.

I want to point out here that there is discussion about dosing or exposure of vaccines to animals. You can gavage a three-day-old rat. You have to be good at it, but you can do it.

In any event, then we looked at this time line, looked at the variety of immune function assays, and I'll show you those right now. This is four weeks, actually, four weeks of age. And this is just basically four days after the last exposure of these pups to the chemical. This is DOTC. You see that we get dose-related suppression of all the mitogen-stimulated responses, the T cell mitogen responses

and the B cell mitogen response. So this is just four days after the last exposure.

We still see this suppression up to seven weeks. Okay? So now we're talking three weeks post last exposure. So these animals still have a suppressed response, as measured by the mitogen responses here.

After that, we checked them again at ten weeks, and they had returned to normal. So this is not a persistent suppression, but it's a somewhat long-lived suppression, at least for these functional end points.

With TBTO, we found effects on the NK cell activity. Here we used two different targets: the yak [ph], which is used primarily--It's a mouse lymphoma; and the WFU, which is a rat lymphoma. And basically found effects at four weeks, which is three days after the last exposure. However, subsequent to this, there are no effects on the NK activity.

These are the mitogen responses. And we also included a mixed leukocyte reaction here. This is three days--four days after the last exposure. Basically, another dose-dependent type of response and suppression of mitogen response and the mixed leukocyte response. We went up to ten weeks, and we still saw suppression here; only at the high dose, however. And again, this is a bit more long-lived an effect than with the di-N-octyltin dichloride.

So just as a summary--I'm not going to go through this, but I just want to point out that we did expose adult animals. These are all done in male animals, by the way. We did do the same dosing regimen with the adult males, and did the different tests, and found no effects whatsoever at any of the doses that we used. So obviously, the developing immune system of the rat, exposed to either of these two organic tints, caused "immunosuppression."

The next group of slides that I'll show you are TCDD. We're looking at a single exposure to TCDD, or dioxin, on gestation day 14, and how that affects the immune system. TCDD is a known immunotoxicant, as I said. There's a lot of work that's been done with it--and actually, work prior to what we did here--by Vos and Faith and Jack Moore, that demonstrated that this is a developmental immunotoxicant. And we decided to look at it a little bit more closely. So basically, we look at--This is a time line, basically. We dosed the animals, the pregnant animals, on gestation day 14. This is by gavage.

We looked at phenotype. We know that there are changes in T cell populations, a block between the double-positive CD4/CD8 to the--double-negative to the double-positive CD4/CD8 in these animals.

And then we looked at a host of different immune function assays. And what we found was that the DTH response was

one which caused effects up to 19 months of age. And let me just show you those data.

Okay, what we did, this is a cross-fostering study, talking about dynamics, pharmacokinetics, and metabolism, and that sort of thing; although this is not a metabolized chemical. With the control we have no effect. This is a dose of one microgram-per-kilogram on gestation 14. Placental: There is placental transport, but we don't have a change in the response. Lactational exposure only: We know that TCDD is found in the mother's milk. No effect. But when we look at the placental and lactational, we get a suppression. This is animals that we did dose response here, looking at how low could we go to see an effect on the developing immune system. This is the DTH response, I'm sorry. The previous slide is the same.

Basically, the DTH response was the most sensitive response, and so we focused on this. Basically, what happens is that at four age you see a dose-related decrease, but it's not significant. However, when you get out to 14 months--I'm sorry, 14 months of age--we had across-the-board suppression of the DTH response. We also looked at a higher dose, 3 microgram-per-kilogram. And this is the data that goes out to 19 months of age.

More recently, we've looked at the effect that TCDD given on gestation 14 has on the DNFB ear swelling response in the rat. And as you can see, at two months old, there is

an effect, at 3 micrograms-per-kilogram; and again, at four, an effect.

The interesting thing, we did this with both BSA--The data I just showed you was with the BSA adjuvant. The other antigen that we use is KLH. And we found the same kind of effects with the KLH-sensitized animals.

This is data from Fan et al, 1996, in which they looked at the suppression of the DTH response to KLH in animals exposed to TCDD. It took a dose of 90 micrograms-per-kilogram to cause a decrease in that particular response. So we're talking about at least over a tenfold difference--a hundredfold difference--in the dosing where we're going to find an effect in a developing animal, versus an adult animal, using TCDD as the toxicant and the delayed-type hypersensitivity as a metric.

Okay. This is just a summary of this; again, highlight the work by Fan. And here is a computer here. This is the KLH adult study where the DTH took 90 micrograms-per-kilogram to suppress the response.

All right. This is a schematic of a group of studies that we did with the National Institute of Environmental Health Sciences primarily, orchestrated by Bob Chapin [ph], a developmental teratologist.

In the early '90s, the National Research Council, under the auspices of the National Academy of Sciences, wrote a document--and the title of that document was "Pesticides in

the Diets of Infants and Children"--because of the concern for children being potentially more susceptible to exposure to different types of pesticides.

And so what we did was, we developed a dosing scheme and testing scheme, that is illustrated here. I know it's real busy, because it has not only the immunotox, which is here, but also the developmental tox, and repro-tox and neuro-tox. But let me just focus on this part here for the immunotox.

Basically, we did the dosing starting around gestation day 14, and in some cases on gestation day 12; dosed the dams; continued to dose the dam for the first week, so that the pups were exposed via lactation. And then after that, we directly dosed the kids. And the reason why we dosed the kids, because this would be closer to what would be happening in young children.

And they're still getting it from the dams. The dams are no longer dosed, but they still have some of this whatever pesticide in the milk, if it is in the milk. And then, we stopped at six weeks of age; we wait two weeks; and then we look to see what happens.

We did five different pesticides. We did carbaryl: Found no effect there. We did tebuconosol [ph], which is a fungicide: No effect there. We did chlorporophoz [ph] [inaudible]: No effect there. However, we did effects in

methoxychlor and in heptachlor, and let me just show you those data.

These are nine-week-old male pups that were assayed for their response to sheep red blood cells. And you can see that there was a dose-related decrease in the antibody response to sheep red blood cells at the very lowest dose and the mid dose here.

We didn't have any other animals that we could use to look further into other immune function end points, unfortunately. So that had to wait for the work with heptachlor.

Now, the heptachlor work is interesting in that heptachlor is no longer used as a pesticide. It's banned in the United States. However, there was an incident in Hawaii in the late '70s and early '80s where heptachlor was used to control mealy bug on the pineapple plants. And as is the case in a lot of agricultural endeavors, the pineapple plantation owners were interested in using every part of that pineapple plant.

Consequently, what they did was they took the leaves from the pineapple and basically shredded them up, and added it to what they call "green chop," which was fed to dairy cows in Oahu. It was only in Oahu. And what happened was that the cows' milk was contaminated with heptachlor, obviously. The doses that we chose here were based on a low dose of 30 micrograms heptachlor per kilogram per day, in dosing these

animals. The reason being that that dosage was within the 95th percentile of the amount of heptachlor epoxide--which is the major metabolite of heptachlor--that 95th percentile of what was found in mothers' milk on Oahu. So these data are relevant, from that standpoint, in this heptachlor fiasco, if you would.

This is just some pharmacokinetic metabolism information. Basically, the blood, thymus, and spleen had about pretty much the same levels. Obviously, the fat had a lot more, because this is a lipophilic, organochlorine compound so you have a lot in the fat. And because it's in the fat it's of concern because if these animals were not exposed post-natally, as the pups were being breast fed they would continue to be getting that heptachlor epoxide.

What we found here, this is the antibody response to sheep red blood cells in eight-week-old mice. This is two weeks after the last exposure, and we see a nice dose-dependent decrease at all doses that we examined.

And then, 26 weeks later, now we're talking about basically 20 weeks after the fact. The IgG response: The same antigen was reduced, as one might expect; but not necessarily expect it to be as "persistent" as it apparently was.

We also looked at the DNFB response. And I must mention, for all of these--for the TCDD work and for this work with the pesticides--we looked at both males and females. It's

an important consideration, given that what we're finding is that males seem to be more susceptible than females. Why, I don't know.

But basically, this demonstrates the suppression of the DNFB response, ear pinna swelling, in the males that were exposed to the lowest dose, to the highest dose.

Again, this is just a summary of what I just showed you. But I want to point out that we looked at the dams. Now these are the females, so they're not going to be as sensitive as the males. But we looked at these females, and we saw no effects after weaning.

What we're doing now is we're trying to dissect the developmental sequence, those periods of developmental susceptibility; dosing the animals during those periods to find out if there is in fact one or two, or maybe many, critical periods of development that would be affected by exposure to this particular pesticide.

Now I want to talk about something that FDA is interested in, and that's drugs. It has nothing to do with vaccines. But this is work from three different laboratories.

The first one is diazepam: Work by Schlumpf et al; did a lot of work with this; used the rat. And in their studies they used both males and females; no real distinction between males versus females.

But nonetheless, a subcutaneous injection on gestation day 14 or 20--of the dam, obviously--at 1.25 milligrams

diazepam per kilogram. They demonstrated decrease in T cell responses, ConA, and mixed leukocyte reaction; decrease in the plaque forming cell assay to sheep red blood cells at eight weeks; alterations in the ability of spleen cells, macrophages, and thymocytes to produce different types of cytokines--the TNF-alpha, IL1, IL2, 6. And this is in four- to six-week-old animals.

And finally, kind of the real acid test for an immunosuppressant is what happens when you challenge it with an infectious agent. And they found suppression of the T.spiralis infection in eight-week-old animals.

I apologize for all these computers and signs I don't recognize. Must be a different version of Power Point, of something.

Dexamethasone: A steroid. Bakker did a lot of work with this. He has several papers, but this paper in 2000 from the JI indicates that there are increased signs of guinea pig myeloid-based protein/complete Freund's adjuvant induced neurological tail tonus and paralysis and hind limbs of these animals. So it's somewhat of an autoimmune type reaction that was demonstrated with the dexamethasone. Also, there were changes: Down regulation of certain types of cytokines, LPS-stimulated cells and ConA-stimulated cells; decreases in a variety of different cytokines. And also, an increase in spleen production of TNF--and I

believe this is gamma, Interferon-gamma, and IL2, at nine weeks old.

So what you have here is kind of a mixed bag of both: an autoimmune type exacerbation of a response to the protein, and some indications for immunosuppression as well.

Acyclovir work, from Stahlmann's lab, using 10 milligrams per kilogram; and this is gestation day ten; subcutaneous injection, either once or three times. And basically, changes in body weight, so there's some toxicity, overt toxicity obviously, associated with this exposure; but decreases in thymus weight in males and in females.

Again, the test with the T.spiralis, trichnospiralis [ph], looking at decrease in the infection, protection against this particular parasite, as well as decreases in the antibody response to that parasite.

Now, finally I come to the human situation. And these are epistudies that deal primarily with organochlorine chemicals.

In Canada, Dewally did work with Inuit Indians in Quebec Province. These are subsistence hunters and fishers, and they are eating wildlife and fish that are highly contaminated, with a variety of PCBs in particular.

And so what they did was they looked at possible problems in the young children born to the mothers of this particular group--this tribe, I guess you would call it.

And what they did was, they were able to associate levels

of DDE, hexachlorobenzene, dieldrin, as measured by the amount of these different chemicals in breast milk, and associate that with an increased risk in otitis media. And then also, they found that that also included the hexachlorobenzene and dieldrin. And this is in one-year-old Inuit newborns. And the population that they studied was 171. So what that says is that these particular children are suffering from otitis media more so than children that are not--based on the levels of these different chemicals in the mothers' milk.

PCBs and TCDDs work was done by Weisglas-Kuperus. This is from The Netherlands, work from The Netherlands. This is a cohort that's been studied for many years now. In the last iteration--It's not really the last, but in 2000--it was published.

Maternal cord blood and plasma and milk, served to the surrogate for the pre- and post-natal exposure to these organochlorine chemicals. They found an association with exposure to both of these types of chemicals, with a decreased antibody response to mumps and measles; again, an increase in otitis media and chicken pox; and then a decreased prevalence of allergy in 42-month-old animals--children, sorry, 42-month-old children.

This change, this decreased prevalence of allergy, may have something to do with a TH2/TH1 shift. They haven't

examined that, but that may be what's underlying this decrease in allergy.

Finally, work by Karmaus--and this is from Germany--looking again at PCBs, DDEs, and hexachlorobenzene: They're looking at whole blood levels of these chemicals in the children that were examined. And the children were eight-year-old children, 340.

And again, what we see is another predilection to increased risk of otitis media. In this case, unlike for the TCDD-PCB work, asthma increased, as opposed to decreased prevalence of asthma or allergic type responses. But there was an increase in IgE. And that's in the seven- to eight-year-old children.

So what we have here are some examples of what can be associated with some of the effects that we see in the animals during the development of the immune system.

So what I'd like to do, to just summarize here: We've used the rat as a model, because the rat is the model primarily for toxicity testing. I think it's a sensitive species, rodent species, for identifying developmental immunotoxicants following either pre- and/or postnatal exposure.

The immune function that we looked at--innate and specific--can be successfully assessed from pre-puberty throughout life.

Alterations initiated during immune system development in the rat may occur at lower chemical doses than those required in the adult.

With certain chemicals--and here we're talking pretty much about the organochlorines and diazepam--it appears that males are more profoundly affected, which may be linked to perturbations in the endocrine-immune network.

Selection of the immune developmental periods for chemical exposure if possible should be based on the pharmacokinetics of the chemical; as I showed with the trans-placental and lactational exposure to TCDD, versus what happened with the organochlorines where it's not passed either via the placenta nor the milk of the dam to the pups.

And from our standpoint, I think it's important--These are all screening now; this is not trying to get to the bottom line of how is this all happening. But for screening purposes, I would recommend that dosing encompass the in utero period, lactational, and pre-pubertal periods of development; basically, loading the deck, if you would, to try to identify potential immunotoxicants, from the standpoint of environmental chemicals.

Thank you.

[Applause.]

DR. SMIALOWICZ: Any questions? Okay. Nobody is coming up for questions, so I guess we're going to go eat. Everybody's hungry, I guess.

[Pause.]

DR. SMIALOWICZ: Okay. Thank you.

[Whereupon, the workshop recessed for lunch, to reconvene at 1:15 p.m., that same day.

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

3A DR. SERABIAN: There's going to be sort of a modification in the afternoon schedule as we have it. Basically, what should have been this morning we're going to start with this afternoon, which is topic one, "Study Design." Dr. Mildred Christian is going to give a short presentation. Then we're going to have a question-and-answer session similar to yesterday.

Then we'll go into topic four--because we feel that with those two topics, there's more of an overlap with those two than with the others--which is "Animal Models." And Dr. Barrow will again give a small presentation. Then we'll follow that with some question-and-answer session.

And then, approximately around three, we will end; we'll have a short break. And then we'll start after that with topics two and three; because again, those two, immunological and developmental endpoints, pretty much--There's a bit of overlap there, also. So we thought that was the best way to organize it. Okay.

And let me introduce myself. That might help. My name is Mercedes Serabian. Right now I am with the Office of Cellular Tissue and Gene Therapies, in Center for Biologics.

I just want to reiterate what Marion had stressed this morning. The questions that she put up briefly in her talk

we're going to put up also during these sessions. And the questions do have a bit of overlap, but that's I think important, because it just shows that basically all the issues and topics that we have have quite a bit of overlap and need to be evaluated.

One big thing, though, is that even when they do overlap we're going to try to keep the session moving and the topics moving as much as we can, just to keep the afternoon moving along.

I just want to stress that, again, the ultimate goal of today's session is to present the guidance document, as was done, and the questions that both we and industry have had at this point; and to try to come to some type of consensus as to the questions and the revisions that we think need to be made to this document. And I think that's really crucial. And it is crucial for you all, as you are the manufacturers as well as the companies that test these agents. Okay.

Let me introduce the first speaker, then, which is Dr. Mildred Christian. Dr. Christian obtained her Ph.D. from Thomas Jefferson University, in developmental anatomy, and has been active in regulatory toxicology for more than 35 years.

After 14 years as a teratologist/toxicologist with McNeil [ph] Labs, which is a J&J subsidiary, she founded Argus Research Labs in 1979, Argus International in 1980, and the

Center for Photobiology at Argus in 1989; at each of which she served as chairman and president.

She merged two of these organizations with TSI Corporation in 1991, becoming vice president of the TSI in vivo testing group of five CROs. Beginning with Genzyme [ph] Transgenics' acquisition of TSI in 1996, she has served as executive director of science and compliance for GTC's Primedica [ph] Corporation, after the purchase of Primedica by Charles River, until November 2002.

In this position she was responsible for scientific integrity and regulatory compliance for the CRL-DDS laboratories, coordinating the product management across the labs, and for reviewing protocols and reports generated by Argus Research.

Mildred has been personally involved in the evaluation and submission of over 1,200 developmental, reproductive, and general tox evaluations, interacting with more than 350 pharmaceutical, chemical, and consortium organizations supporting these activities.

She has also developed more than 1,000 position papers for chemical and pharmaceutical companies, the FDA, the EPA, the Office of Technology Assessment, and the OECD.

She has also been involved in the ICH repro-tox guidance documents, the "red book" document, and many, many other numerous documents that I don't have time to present at this point.

Dr. Christian.

[Applause.]

STUDY DESIGN

PRESENTER: MILDRED CHRISTIAN, PH.D.

EXEC. DIR., RESEARCH, ARGUS RESEARCH LABS

DR. CHRISTIAN: I will make a statement that sounds like I'm with the government now. These are my own opinions that will be presented, and not those of anyone else. The designs that will be described are those which we used in studies over the years, and they represent to some extent the development of the procedures in testing for these types of compounds.

The basics are that when one does these types of studies, as mentioned yesterday, they of course are performed in conformance with GLPs. That's basic.

Then we're supposed to have them do the route and frequency of administration that is mimicking clinical use. Sometimes, very difficult.

Consider the pharmacokinetics: Well, that's perhaps relevant to the adjuvant, as we heard yesterday, but not necessarily to the active portion of the compound; the pharmacodynamics, though, certainly, of these vaccines. Bioavailability--this is something important; the volume that can be administered. And then, identify dose-response relationships, something we've heard may not be too important, or even relevant, with these types of compounds. The reason I say that--and these are the considerations as compared with the basics--is that we're going to look at

only one species--theoretically, the relevant species--which we did a great deal of discussion about, and will do some more later, as to what is relevant.

Clinical use: The clinical use is really that we are, at least in theory, addressing the immune response; which is quite different from the classic developmental toxicity study in which one would address the response to a drug or to a chemical.

And then, we are also looking at the potential toxicity of at least two components; one being the vaccine itself, and the other, the response to the vaccine with an adjuvant, and possibly of the adjuvant alone.

When we were developing the ICH guidelines, this is what we came up with. Now, these are the segments. And when you see reference to the ICH guidelines for reproduction and development, what is important--and one of the reasons there's some confusing nomenclature perhaps used--is that reproduction is the whole cycle. And it starts with reproduction, conception, and you go all the way through, and end up with maturity, the next generation, and sometimes go into senescence. And what we said for the ICH guideline was that we were to look at each segment.

Now, what is come up with for these types of testing was when the initial thought--And this was really something that Joy Cabanero [ph] and I worked with many, many years ago. The initial thought was--because no testing at that

time of repro-tox--Would there be any effect of the immune response on development? And would that possibly cause the most expected changes in the endpoints: abortion, death of the conceptus, malformation, reduced fetal body weight?

So we were at that time thinking strictly in terms of the type of developmental toxicity that is usually evaluated in a developmental toxicity study, which ends at C-section.

Do you address function? No, because you don't look. They're dead. Do you address immune response? That wasn't normally done. But remember, what we usually did was we had to dose every single day of gestation, because every day is a moving target in the developing conceptus.

And so the normal developmental toxicity study starts about implantation; goes through embryogenesis, with exposure there, and that being the period most likely to result in malformation. After palate closure, during the fetal period, that's the period of growth. And generally, these two "C" and "D" sections, as you heard earlier, are the intervals that one is concerned about in a developmental toxicity study.

However, you've also heard that we should do boosters; we should do it at the time of peak response. And that results actually in having a study that starts pre-conception. And so we do do some evaluation of fertility already in the design, if we do the booster shots.

And to look through to weaning has been suggested, and that would certainly be some postnatal evaluation; although not necessarily, as I'll show you, sufficiently long to see if we had immune effects out late in life.

This is just a summary of some rather large points, to show that the human and the mouse, at least, are not the same. And we've gone over that several times. But I think what is important here is, if we are attempting to maximize response in the rodent species, it's really in the fetal and postnatal period; and it's in the first and second trimester in humans.

And this is a repeat of that showing in a mouse or a rat, with the maturation with immunocompetence going on one year; 30 days postnatal. Immune memory, going up to 18 years in humans; mouse or rat, 30 to 60 days postnatal. So we have different time points when the targeted tissues might be sensitive.

Now, what is the response of species, and when is the maximum response? If we look and take the concept that the maximum immune response should be present during the most sensitive period of gestation, classically that's usually considered the first trimester for morphologic changes; and need to initiate treatment before if we need a booster. But we also have to remember we're going to give several injections. And ideally, we'll have to have information obtained about when we need to give those injections from

at least non-pregnant animals, so that we can compare them with pregnant animals and see if pregnancy itself is something we need to be concerned about. That is generally not done.

Now, we all know certainly there are several components for vaccines. We should know the general toxicity of each of the components. And for the adjuvant, I think at the very least there should be an arm in the developmental tox study, if it is a new or unusual adjuvant. I'll show you what I mean by that, and why.

We have heard that the most common dose tested is one times the human dose. And in the studies which I'm going to show you, they were generally done for NIDA. And there was a series of them that were done based on when the maximum immune response would be reached.

There are also some that are proprietary compounds, and they were similarly either studied ahead of time, to find out when the maximum response would be present, or dosed sequentially with different sets of animals, so that that could be evaluated post-testing. And then one could look at when the maximum immune response was present, and identify which group was considered the most relevant for testing and evaluation.

It has to be remembered that sometimes the doses are limited by local toxicity. And that's very important in developmental toxicity because of the secondary effects of

local toxicity. We know that if we were doing a dermal study and we caused remarkable irritation to the dam, there are certain things we would expect to happen. We'd have stress reactions that would result in secondary effects in the fetuses. Most likely, we would see such things as extra ribs; we might see some reduction of fetal body weight; we might expect to see some increase in resorption. We also know that if we're dosing before implantation and we have stress reactions and a boostered immune response, we may get a lower incidence of implantation. And for that reason, when we're doing artificial insemination in rabbits, or natural mating, with prior treatment, we add more animals to the study, simply to ensure that we have sufficient numbers that become pregnant for evaluation. Often, more than one dose in the series of studies I'm going to show you that we performed; but seldom is there even an attempt to show a classic dose response. And that's appropriate.

How many doses are generally tested? That's certainly on a case-by-case basis. And it would be dependent not only on the onset of the response, but also on how long it lasts, the pharmacodynamics of the compound.

And then, of course, the effect of boosters. Whether it increases the response, maintains it, or whether it's going up and down during that whole interval, is important.

Now, the developmental tox endpoints to look at, I would think, certainly would be, at a minimum, the classic ones, but would go through birth. Why? Because the immune system, if that is one of the target organs, isn't going to be even partially developed to an appropriate extent until postnatally.

This is just my own impression: Unless there is a particular need, I would not add in crown-rump length because it's a very insensitive parameter, in that it's highly variable, particularly in rabbit species. It's a little bit better in small rodents.

Organ weights: I put in I don't know that they would be necessary. They are highly variable when there is a selected number--and that number, if there's only one or two per litter that are taken. And of course, because it is a developmental tox study, the litter would be the representative unit.

And we found in our laboratory, unless we have at least three on average on the basis per litter for males and females, that the organ weights are not truly representative of the litters, and that statistical analyses are often misleading, both as false negatives and false positives. So I would recommend, if we're doing organ weights, to do at least three per sex per litter.

Antibody levels can be looked at for the mother, for the fetuses, and should be looked at for the pups. And this

would answer the relative questions about: Is it present, and does it persist? I don't think doing the whole kinetics as an initial screen, in the absence of other effects, would be appropriate.

One thing that must be considered is not only the immune response, but is the potential for antibody transfer present? And that is dependent on the placenta. Exposure in the conceptus may not be the same as it is in humans. And for that reason, we chose, when we were initially putting some of the study designs together, to use rabbits. Because placental transfer in the rabbit occurs, antibodies do cross, and it's much more similar to what happens in the human placenta than certainly the rodent. Or we've been asked sometimes to even do canine studies. And you must remember that certainly even a pig, it doesn't cross at all. And you'll be hearing more about species differences later.

Timing differences: Theoretically, we're to use the species--and this would be for any developmental tox study--the species with the best response, and with placental passage, and with the immune system most like humans.

And Paul will be talking a little bit later, but I'd just like to show you here. We do guinea pig occasionally, because of the longer time in utero and the comparable development of the CNS in the immune system to humans; not completely comparable, but both guinea pig and pig, closer.

Rabbit: Quite a bit postnatal. But it has two of the things: it mounts a good immune response, and you have placental passage.

Mouse: Maybe not. Most of the immunotox information there, but not quite as good a model.

We've done ferrets. One had a canine. Only responsive species.

Non-human primate: Perhaps. Very good, but very expensive, and limited in numbers; so not always the best model.

This is a summary of the study designs I'm going to show you. You'll notice that they were done either when their maximum response was present, or they were given at various times during gestation.

In all cases, they checked for placental passage. The rat was usually intramuscular. One test group generally at one times the human dose. Whenever there was a new or unusual adjuvant, it was tested as a separate arm. Most included shots that brought up injections pre-mating. And this gives us some indication of potential effects on the female fertility.

Some of them were followed postnatally. And the observations that were made there were generally for viability; growth; nursing activity, which in the rabbit is a very good measure of whether it has normal behavior or not. One has to remember, there is a certain number of

rabbit mothers that don't like their babies, so you need some background as to what is the normal incidence of pup loss. And antibodies were looked at, both in the does and the pups.

When you're on maternal effects, something that should be remembered on a practical basis are daily observations. Because when one is injecting or administering a compound at weekly intervals, you want to follow the pattern of effect for developmental toxicity anyway, because the later days of the gestation may be those where the effects are. So if the injection is given on day one, the next day the mother may not eat, may lose weight; and you'll see a weight loss, and a weight gain. But if you only weigh weekly, you'll miss that. And if there are any effects on development, it wouldn't be seen. So even when the injections are given at weekly intervals, there should be daily body weights in your developmental toxicity study. And here what we did find was when there was daily treatment--And we have a study with daily treatment. Why? Because every day of gestation the sensitivity of the animal changes towards the response that will occur, or potentially occur, in a conceptus. We found that there were effects on the dams that were not observed when there were fewer treatments. It's not remarkable; it's just something that one should be aware of.

We also found that the only studies in which we saw adverse effects on embryo fetal development were those in which the adjuvant arm showed similar effects.

This is a study design in mice. It was a developmental tox study, which meant treatment was limited to one week pre-mating, or gestation-six, or gestation-13. Why? Because that got at least one treatment during embryogenesis, one treatment that would occur over fetogenesis. And the dose was two times the human dose. We saw no effects in either the dams or the conceptuses.

This is a group of rabbit studies. In the range finding study, this is the longest one we had. Six weeks pre-insemination; three weeks pre-insemination. It had been already determined it was a three-week period to reach maximal response with a booster. And then during gestation, gestation days six, 12, and 18, with the vehicle alone, a high dose.

These animals were determined to be sero-positive at two weeks, and only those that were were continued on study. Doses at 1 and 2 "X." There were samples. They were worried about immune complexes. The kidneys were weighed. There were no effects on the dams or conceptuses.

This is a developmental tox study with daily dosing, seven to 19; a control; an adjuvant; a low and two high doses, one at the high dose of 20 times the human dose. This is one of the NIDA studies. It's a compound that has been

used; it's a tetanus toxoid. It had been in use in humans. Two high doses, one which followed the seven and 19, and one which was seven and 12 and 18 during gestation. Here we had maternal toxicity in the daily dosing. No developmental toxicity in either daily or the weekly dosing during gestation.

Another developmental tox, beginning four weeks. And this is the weekly schedule, four, three, two, and one, pre-insemination. And then another dose on gestation day 18. These were samples taken of antibody levels. They were taken for the mother for baseline level before the first dose at two weeks, at four weeks, gestation days 18 and 19, from the fetuses at C-section. Antibody titres were present. No effects, or no adverse effects.

Another IM study: One week pre-; different schedule, two, six, and 13. In each case, these are based on predetermined information as to when the maximum responses were present. A placebo control, and three high doses, day two, day six, or day 13. And the mothers were bled, and antibody levels determined before the first dose and on GD-29, which is the day sacrificed, so that they could figure out if there was persistence or when the peak effect occurred.

This is another one: Two groups, IM. The difference is, you can tell the number of samples that were taken. This is the first set that would go postnatally. And there is

another set that is taken at lactation day 21, when the animals were weaned. Four weeks, one week, sort of the standard after that--seven, 14, and 24. One times the human dose, which was 20 times the maximum human dose. Fetuses at gestation 29; pups at lactation day 21; the mothers before each.

And the others are quite similar here, going the same way. But the important thing is we were making these determinations.

This is a ferret study, selected because that was the responsive species. A quite large study. Treatments were days three, six, 13, or 22. A vehicle and a high dose at one times the human, so there would be a vehicle and a high at day three, at day six, at day 13, and day 22. Samples were taken at termination on day 35.

What can we say about this? Well, most studies, the evaluations were limited to the immune response. The antibodies were studied in the dam, the conceptuses, and the pups, to determine either before or after what the peak levels were. Most looked at only one dose.

I think this is important. Most did not administer the test material or get a peak response in the animals during the period when the animal's immune system was developing. Because the purpose wasn't to look at the target of the immune system; but rather to see if we caused death, abortion, or malformation.

I believe that's very important. My personal opinion is, if we're going to consider the immune system the target, we'd better consider treatment postnatally of the mothers and seeing if the antibody comes across in the milk and if they continue to be exposed to it during the lactation period.

To date, no study we conducted with these types of vaccines looked at potential effects on the immune system. However, when we have used other types of vaccines and immunosuppressive agents, we have seen immunosuppressive effects that were not evident until after puberty.

And I think this is important. None compared the pregnant versus the non-pregnant animals. And if we are worried about the offspring, we should also be worried about the pregnant animal potentially being different from the non-pregnant animal. And the same applies, I would hope, to the pregnant woman versus a different potential sensitivity.

We look at potential effects on embryo-fetal development, but it's really only regarding the presence of antibody. The transfer and persistence can be addressed by looking at fetal levels and pup levels, and at least knowing whether it persists in the pups up until weaning. But if we want to look at immune function, the designs do have to be changed as FDA has suggested.

He says "No," but I think the EPA data also support that.

Viability and body weight and growth are the best indicators today, 14 postnatal. After that, if it's a rat or a mouse they'll start eating material food; they're on their own; they're weaned; and the whole weight pattern and viability, there's a second dip in viability.

Dose response: The only dose response we saw were effects of adjuvant. I haven't showed you all the studies we've done, but just gave you some samples.

I think fetal tissue interactions are probably unnecessary, but possibly indicated on a case-by-case basis.

I don't think histopathology would be remarkably additive to the quality of this study, and would be only indicated if there were effects on organ to body weight ratios. And it must be remembered that, to have any value of them, we need at least three males and females per litter, and it should be evaluated on a litter basis.

So that probably gives you enough to think about. And I thank you for your time and the opportunity to show you some of these designs.

[Applause.]

DR. SERABIAN: Thanks, Millie.

We're just going to switch slightly, and I'm going to ask Dr. Barrow to give his presentation now. And then we'll combine those two topics. I think that's a much better use of time at this point.

Dr. Barrow studied in London, while working at the same time for the reproductive toxicology department at Beecham Pharmaceuticals. Over the last 19 years, he has worked for Cieros [ph] in Italy and France.

He is an active member of the American and European teratology societies, and is a frequent guest lecturer at faculties or facilities in Paris, Lyons, Strasbourg, and Toulouse.

Paul is presently director of toxicology at MDS Pharma Services Preclinical in Lyons.

ANIMAL MODELS

PRESENTER: PAUL BARROW

DIRECTOR OF TOXICOLOGY,

MDS PHARMA SERVICES

DR. BARROW: Thank you for that introduction. I'm very pleased to be here.

As a lead-in to the next discussion, I'd just like to give a rapid overview of some of the considerations that I consider important in species selection for developmental toxicity testing of vaccines. At the same time, I'll give a very rapid overview of some of the work that we've done at MDS on behalf of Aventis Pasteur of four new vaccines presently in development.

So we can start with the obvious question [Shown on Slide: "Which is the Best Model?"]. Every regulatory toxicologist hears this question at least twice a month; not only for

vaccines, but for practically any therapeutic carrier you might think of.

And strangely enough, the reply is nearly always the same [Shown on Slide: "It's the Primate, Stupid!"]. Of course, the best model species is going to be the primate, for all developmental toxicity studies, or practically all.

It's worth remembering at this point that the very first regulatory guidelines were issued by the FDA back in 1966. And this was a direct response to the thalidomide tragedy. Thalidomide, as it turns out, is practically only teratogenic in primates, at least at human therapeutic doses.

However, even back then we decided--Well, that's the royal "we"; I was seven years old. Even back then it was decided that we would use rodents and rabbits for our routine developmental toxicity screen.

And the reasons for this are just as valid today as they were 40 years ago. There are just not enough primates in the world to supply our routine needs for routine developmental toxicity testing. And this situation is getting worse, not better; with practically all Western governments being very reluctant to license new primate breeding facilities on their soil.

To make matters worse, to get a valid developmental toxicity study in the primate we need to use relatively high group sizes. To start with, each monkey normally only

has one fetus per pregnancy. And also, primates don't tend to reproduce well in the laboratory. They have a high abortion rate of around 15 to 20 percent. So in a typical primate study, we're lucky to obtain ten fetuses per treatment group to examine at the end of the study; as opposed to 200 more per group in a typical rodent study. One other disadvantage of primates which is particularly pertinent to vaccines is their long life span. If we want to expose primates pre- and postnatally, and then look at the functioning of the adult immune system, we're going to have to wait four to five years. Now, I don't know many of you out there that have that sort of patience.

So what are the most likely alternatives? Perhaps we won't have the choice. Perhaps the vaccine is only immunogenic in the primate, in which case we can't justify other species.

The three most obvious alternatives are the rat, mouse, and rabbit. Although, after listening to Millie's presentation, I should have added the ferret and the guinea pig to that list. I haven't done that, because I haven't used them personally.

The rat is the most frequently used species in developmental toxicology. Also, we heard this morning that a lot of developmental immunotoxicity work has been done in the rat.

Having said that, there's no reason why we can't use the mouse. Anything we can do in the rat is also perfectly feasible in the mouse. The mouse also has the advantage of having the most studied immune system of any animal.

I should also have said that the rat is often the only species in which we do postnatal examinations for developmental toxicology studies with drugs.

The second most used species after the rat is, of course, the rabbit. But the rabbit is normally only used for prenatal toxicology. We don't normally do postnatal examinations in this species. As Millie said earlier, postnatal examinations are very difficult; although we can't always avoid it, as you'll see in a moment. And as we heard yesterday, a lot of immune tests are not valid, or simply not available, in the rabbit.

Here are some of the considerations that we bear in mind when choosing a species. Evidently we want to choose a species that does mount an immune response to our vaccine; bearing in mind, of course, there may be quantitative and qualitative differences in immune response between species.

One point raised in the FDA draft is the timing and rate of maternal antibody transfer. I'll come back to that in a moment.

And also, we're going to want to be able to do both fetal examinations and postnatal examinations in our chosen species.

Coming back to maternal immunoglobulin transport, as we've heard, the big difference between primates and rodents is the timing of maternal antibody transfer to the offspring. In primates practically all maternal antibody transfer is before birth. As it turns out, according to the literature at least, this is also the case for the rabbit and the guinea pig.

In rodents, however, only about 10 percent of maternal immunoglobulin transfers before birth, with the other 90 percent transferring across in the milk or the colostrum. And other species, as it turns out, are even worse, with little or no maternal antibody transfer before birth.

Now, this is the strategy that we have used to test four new vaccines. We normally start off with preliminary studies to look at the maternal immune response in the pregnant animal, and also to look at the timing and rate of maternal immunoglobulin transport, in each of three species: the rat, the mouse, and the rabbit.

And on the basis of these results, we normally choose just one species, to go ahead and do the main developmental tox study. We normally hope to be able to use a rodent because, as I said, the postnatal examinations in the rabbit are very difficult, although we've not always been able to avoid this.

So in the preliminary study we start with groups of 12 female animals of each species--rat, mouse, and rabbit.

I've gained some new characters here. I didn't make that choice of bullet point. I think these are probably the characters that were missing from Steve's presentation this morning.

We treat animals of all three species before mating, according to a predetermined vaccination schedule which is based on the known immune response in that animal, and also on the proposed vaccination schedule in humans. So in a typical study, we'll treat the animals two or three times before mating, at ten-day intervals.

After mating, we then give all the females a booster vaccination on day six of gestation. This serves not only to maintain high maternal antibody levels throughout the remainder of gestation, but also hopefully to expose the developing embryo to the actual components of the vaccine formulation.

Six females--that's half of the females of each species--are then sent to caesarean examination, where we take blood samples to look at fetal titres and maternal antibody titres.

The other six females of each group get another vaccination at the end of gestation; are then allowed to give birth. And we kill off the females and pups on day 11 post-partum. Again, we take serum samples to look at antibody titres in the pups and mothers. The FDA suggests that we also do

antibody analysis in milk. Unfortunately, we've not been able to do that so far, because of analytical difficulties. This is an example of the type of results we obtain in this preliminary study. The blue blocks are fetal antibody titres. The red blocks are antibody titres in the pups on postnatal day 11, and these are expressed as a percentage of maternal titres. This was with an HIV vaccine.

We see here in the rat, fetal titres didn't reach maternal antibody levels before birth. In the mouse however, we did get a good prenatal transport. So we were able to justify the use of the mouse with this particular vaccine. As expected, we also got a good prenatal transport in the rabbit. I would also note that in all three species we did get a good persistence of maternal antibody levels in the pups up to 11 days of age.

So for the four vaccines tested to date, we were able to justify the use of the mouse for two of these vaccines: the HIV vaccine, and the tetanus/diphtheria/whooping cough vaccine.

Unfortunately, in two of the cases, we had to resort to using the rabbit. In the case of the meningitis vaccine, this was because of poor or unpredictable immunogenicity in the pregnant animal, in the pregnant rodent. But in the case of the rabies vaccine, this was because of poor maternal immunoglobulin transport before birth.

We then go on and do the main study. We use the same vaccination schedule as in the preliminary study. Here we start with groups of 40 rodents, or 35 rabbits. One subgroup of animals goes to caesarean, and we perform all the routine teratology type examinations. The other subgroup is allowed to give birth, and we do all of our postnatal followup on the litters following birth.

This second generation is normally terminated at weaning. Although if we do see any indications of developmental toxicity--which we've not done so far--we will extend the study to cover a postnatal followup, possibly with behavioral examinations, probably adding immune assessments; and perhaps even mate the animals to look at their fertility.

I would just like to ask one question before finishing, concerning comparative development and maternal immunoglobulin transport. I wonder if we've not been a bit misled by this. I wonder if we've not been premature in rejecting the use of the rat.

As we have heard this morning, rodents are very immature at birth, by comparison with humans. For instance, the erythropoietic activity of the bone marrow is already well in place in humans at the time of birth, but continues to develop postnatally in rodents. But we have also heard, nevertheless, the ontogeny of the immune system is fairly comparable between mouse and, I assume, the rat and humans.

My question is: Are high fetal antibody titres really necessary, given that the critical period of immune development in the rodent probably occurs postnatally? And as we've shown, we do get good maternal immunoglobulin titres during this period. So providing there is a postnatal followup, we might not need to ensure exposure of the fetus to antibodies in rodents.

I don't claim to have any conclusions; though I do hope to have some information to fill in this slide by the end of today. So I guess now we just have to put the hand into the hat, to see what we can pull out. Thank you.

DR. SERABIAN: Okay. I think we have about an hour, roughly, maybe a little more, to go over the two topics.

[Tape Change.]

3B DR. GRUBER: My name is Marion Gruber. I'm with the Office of Vaccines.

MS. MILLER: Margaret Miller, FDA, Office of Women's Health.

DR. VERDIER: Francois Verdier, Aventis Pasteur.

DR. INSEL: Dick Insel, University of Rochester.

DR. HOLLADAY: Steve Holladay, Virginia Tech.

DR. SMIALOWICZ: Ralph Smialowicz, the Environmental Protection Agency.

DR. CHRISTIAN: Mildred Christian, Argus Research.

DR. VAN DER LAAN: Jan-Willem van der Laan, The Netherlands, Medicines Evaluation Board.

DR. BARROW: Paul Barrow, MDS Pharma Services.

DR. HASTINGS: Ken Hastings, Division of Special Pathogen and Immunologic Drug Products in CDER, FDA.

DR. SERABIAN: Okay. I think initially we'll start off with--You have the questions in the pamphlet that you got. We'll start off with the first question, just because it's a rather broad question. And please feel free, you know,

with any additional questions, to go up to the microphone stands. So this is just to start us off. Okay?

The first one is: In addition to endpoints outlined in the ICHS5A document, what additional parameters should be evaluated; such as immunological parameters, histopath, and functional assessment? It's what parameters; i.e., if you think functional assessment, what do you mean by that?

DR. VAN DER LAAN: Should we reserve this question to the last round? In fact, it is the endpoints session.

DR. GRUBER: Yes, we can keep this rather flexible. And we will just leave this up there, and we'll just maybe screen through the questions, trying to get some answers to some of them. But perhaps we start off the discussion.

Or if somebody has questions regarding the two presentations that you just heard, then please come up to the microphone.

MS. HELPERIN [In Audience]: Yes, Jane Helperin [ph], ID Biomedical Corporation.

This is a question for Dr. Christian. I was wondering if you could give us a little more information on what compounds you were looking at in the studies you were discussing? And also, with regard to the different animal

models used and the study designs you used, what the basis for that was? Such as, was there any background information or historical information which caused you to choose the designs you chose?

Because I think one of the reasons we're here is to try to figure out what rationale we should be using for study designs. So maybe you could give us a little more information on that?

DR. CHRISTIAN: Yes. With the exception of three of the compounds, they were all NIDA vaccines that were used either for--There was a flu, a tetanus, a hemolophius--Yes, there was an HIV, and an influenza.

And there was background data on each of those that told us the time for the booster shoots and how long it would take to get the maximum response.

All of those studies that were performed were performed for the purpose of evaluating whether they caused abortion or malformation, or affected fetal size in utero. None of them were done as functional assessments of postnatal development of the immune system, because that was not looked at as a target.

Rather, there were concerns whether immunization of pregnant women, particularly in Third World countries--if that would be a problem that would cause them potentially to have problems with morphologic development of their conceptuses.

And so they were designed with that in mind, and without a postnatal phase; other than in, I believe, six of them: evaluation of viability and persistence of the antibodies in the milk and in the pups.

PARTICIPANT [In Audience]: I have a question for Mildred or Steve or anyone who would like to answer it. But it seemed like some of you had looked at thymus-to-body-weight ratios. I always felt that was a very sensitive indicator for developmental immune changes. And did you look at that? And did you find it not to be the case? Or did you just not look at it?

DR. HOLLADAY: For all of the chemicals that I showed you, we looked at them and we really didn't see any effects on thymus-to-organ ratios, or spleen-to-body weight.

DR. CHRISTIAN: We didn't see any, either. But we did look at it in four of them.

DR. SMIALOWICZ: Well, Mike, you and I published a paper together in '96, EHP, evaluating fetal immune parameters and their sensitivity for indicators of developmental immunotoxicity. And of the indicators we found that were most sensitive, fetal thymic cellularity was among the sensitive ones in mouse models. When we correlated those data, they were more sensitive, or that was a more sensitive endpoint than fetal thymic markers, which occasionally didn't change when cellularity went down.

I contrast, cellularity of the fetal liver was a relatively poor marker of developmental immunotoxicant exposure. But marker expression in fetal liver was a pretty good indicator of developmental immunotoxicity.

So the summary of what I just said, according to our review in '96, is that fetal thymic hyper-cellularity is often a very sensitive indicator of developmental immunotoxicant exposure. It will, of course, depend on the chemical that is being evaluated. And fetal liver marker expression, again, is sometimes very sensitive.

I think DES and TCDD are beautiful examples. I suspect that the fetal liver progenitor cell may be the definitive sensitive cell for dioxin exposure. This is an exquisitely

sensitive cell. So TDT positive cells in fetal liver in a mouse: pretty sensitive indicator.

DR. HOLLADAY: If I can make a little clarification here, we never looked at the fetus. We looked at animals that were at least--well, post-weaning. So we didn't see any effects there.

MR. STUMP [In Audience]: Don Stump [ph], World Research. I just wanted to ask the panel what their thoughts are on the designs as Dr. Christian and Dr. Barrow both talked about, immunizing before gestation and then also during various points during gestation.

Any thoughts on whether it's better to take the same group of animals and immunize them before breeding and through gestation; as opposed to taking subgroups where you have some animals that you only expose during gestation, some you only expose prior to gestation?

Because it's certainly differences you might see in terms of giving that vaccine to an animal that has not previously been challenged by the vaccine.

DR. CHRISTIAN: Yes, I think you have to do some range finding or pilot work first, to know that. And certainly, we did modifications based on when the responses were

there. In some cases, we did multiple groups on separate days of gestation because the response--For instance, if we gave it on day six, it maxed about the middle of embryogenesis. And at other times, gave it pre, based on the onset of the effect.

I think it was most effective when given prior to gestation, and the booster given. And it probably had the least effect on the mother. What we were originally worried about when we started these studies--and that maybe was ten years ago now--was the potential effect of fever and its effect on each protein, and what would occur there. And we found that we didn't have any problems with that. That is different from some other types of vaccines. But with these therapeutic vaccines, it wasn't a problem.

DR. BARROW: I don't actually see the point in performing groups that are only vaccinated during gestation, unless we're trying to look at possible effects of other vaccine components other than the induced immune response. We have to treat them before mating in order to get a maintained immune response throughout gestation.

DR. VAN DER LAAN: May I comment also on that? I think it's pretty important the way that Mildred has presented

the different days, the different periods during pregnancy. I think that that might give important information if you take your starting point from the clinical use of the vaccine.

If you give repeatedly a vaccine during pregnancy, that's never resembling the clinical approach. If a woman has been vaccinated before pregnancy, it's not clinical usage to do it again during pregnancy. So the most important problem is when the woman is pregnant, and then to be treated. And that might be important then, to know at which stage during pregnancy.

DR. GRUBER: Perhaps to further consider this point, I think what is apparent and what is important to really do in these studies is to administer priming doses prior to gestation. I think this has been becoming apparent from the discussions that we had today, and presentations. And it's also from discussions that we had when we looked, or when we designed developmental tox studies for these vaccines.

There is one point, or one question that I wanted to ask the experts. We have been recently considering, rather than giving multiple doses to the same group of animals

during the period of organogenesis--let's say, between days six and 18--to really divide the animals into subgroups, and to dose certain groups at certain days of gestation only--for instance, to do it at day four, days six to ten--so that the animal is dosed then only once, or a given group is dosed only once. So of course they have been primed prior to gestation, or prior to conception. And then they receive one additional dose during gestation only. How do the experts feel about this?

And the reason why this is done is because we think that, especially if you look at vaccines targeted for adolescents and adults, many times you don't really give multiple doses to the human target population. So how do the experts feel about this type of design and schedule?

DR. CHRISTIAN: I'll start, and see if it can be controversial. I think the whole problem is the question. And if the question is inadvertent exposure of a woman who becomes pregnant, that's one question. If it is intended exposure, then the design is different. And there are vaccines with extended exposure during pregnancy.

When it's intended exposure, it should be started during the pregnancy, because that's the clinical use, and you

know that the response will be developed during the pregnancy. And one might want to do that then with multiple groups during pregnancy, so that you could see the effect of how long it takes to mount the response during a pregnancy and when it's most effective. And that might be combined with an efficacy study, to evaluate at the same time both the effect on the pregnancy and the efficacy of the treatment.

If it's inadvertent exposure, as might occur when, let's say, we go to a country and just inoculate everyone--And many times certainly there are some countries where the people won't say they're pregnant. That would be against it. So they get inoculated. And now you have all different times of exposure. There it would be probably most appropriate to see the maximum response that can be maintained over the duration of the pregnancy. And a priming dose in that case would probably be appropriate, so that you could build up to the maximum response.

So it's really what question. And that, again, goes to the case-by-case use.

PARTICIPANT [In Audience]: Millie, are you talking priming, or frequency of dose? I guess I'm getting a little confused.

DR. CHRISTIAN: Well, actually, both. You'd want to do it before pregnancy, and then a booster shot to make sure--And you'd have to have some data, probably from non-pregnant animals, to know how to get to the maximum response. Because the question would be: At the time of maximum response, what would be the outcome of that pregnancy?

PARTICIPANT [In Audience]: So basically, potentially a dose prime, and then a single administration at that time point? I'm just trying to understand. Versus several doses, you know, gestation days--

DR. CHRISTIAN: If the question would be--

PARTICIPANT [In Audience]: --six, ten, and 12, or something.

DR. CHRISTIAN: Yes. Would it affect implantation? You might want to do one before--

PARTICIPANT [In Audience]: Separately. Okay.

DR. CHRISTIAN: --mating; then one around the time of implantation; one at the time when peak morphologic development is ongoing; one when there's fetal development.

And depending on the pattern of the response for a particular vaccine, the separation or even the need for additional doses would have to be determined. You know, if you can mount a response that's going to last the entire gestation, then you wouldn't give another shot.

DR. VERDIER: Just one remark regarding the difficulty to scale the vaccine administration, compared to the gestation period. The effect of the vaccine will not be immediate. I mean, you cannot say, "Okay, I will give the vaccine on day six of gestation to evaluate the potential adverse effect at this period of the gestation," because in fact the vaccine effect will last for several days, and will not start immediately after the administration.

That's why it's quite difficult to adjust the vaccine administration with the gestation schedule. And that's why I think we should say, okay, we start--Perhaps we should consider a very large period and say that, okay, we give the product on day six of gestation, in order to cover day six and perhaps the next ten following days.

Unless we want to evaluate the toxicity of one chemical constituent of the vaccine. But I think in this case, that's not the right method. If you want to evaluate the--

DR. CHRISTIAN: That's a different question.

DR. VERDIER: That's a different question. I mean, in this case we have to refer to the ICH5 guideline, and study the teratology of chemicals by normal way. But I think that's not the discussion now.

DR. CHRISTIAN: No. Maybe I was misunderstood. If one knows when the peak response is present, you might have to give it before mating so that for the duration of the most sensitive period, let's say, in a rat, essentially days six to 20, and possibly staying in maternal milk--And going over and being exposed that way. It might be fine to give it ahead of time, if you had that long a duration of response. If not, one might have to give an additional booster shot, or even two, before mating.

And that's why those designs--You notice there was one that had four pre-mating, and it started way out six weeks before mating, because it took that long to build up the maximum response.

DR. VAN DER LAAN: What do you mean with "the maximum response"? What's the most risk-full effect during pregnancy? Is that the existence of antibodies? Is that the transfer of antibodies through the placenta? Or is

that the increase of cytokines, interferons, and all of those other elements?

I have the feeling that we should be aware of where we are talking about. Are we defining the maximum response as the antibody response, or other types of responses?

And that's also a question to Dr. Barrow. In his talk he indicates the selection of species based on the placental transfer of the antibodies.

DR. BARROW: Yes, that's a good question, to which I don't have an answer. Perhaps I could pass it over to another member of the panel.

[Laughter.]

DR. CHRISTIAN: Out of naiveness, like most of immunotoxicology, this is a rapidly evolving field. We don't have--Certainly, I don't have all of the answers. But what I was talking about in terms of maximum response, what we were looking at was maximum levels of antibody production.

Of course, with the placenta we know that the permeability of the placenta, and the passage, and the way it goes across the placenta, change with gestation; with the

placenta becoming more permeable as gestation continues. So that, again, is changing with time.

And you're exceptionally correct with the cytokine production. We are concerned about that, because that would be what would induce a potential response that's secondary in the conceptus. However, whether or not we know what to measure certainly would be on a case-by-case basis, and modeled for that particular compound.

DR. HOLLADAY: I'm speaking from an immunotoxicologist's perspective. But clearly, there are data that different immunotoxicants have different windows of susceptibility prenatally. Chlordane is a good example; lead is another good example.

I think of immunosuppression typically in the work that I do. And in the case of this meeting, what I'm hearing so far, I'm not overly concerned about the effect of vaccines on a postnatal immunocompetence. My thoughts are more in line with, I suppose, exaggerated immune responses, hypersensitivity disorders, possibly autoimmunity.

And I think now about a paper recently that came out by Anser Ahmed [ph], who exposed animals to one low-level dose of diethylstilbestrol prenatally; carried these animals

until they were geriatric. And from all parameters assessed, they appeared normal immunologically, until a secondary DES challenge was given. And at that time it was shown that their cytokine production profile was skewed in a direction that would lead one to predict they might be more prone to develop an autoimmune disorder.

I could almost see that type of thing happening with a prenatal maternal immune stimulation that skewed the fetal immune development such that it could be a very difficult thing to pick up, but in the right person at the right time with the right environmental exposures or combined exposures, we might see a phenomenon like this DES phenomenon. It's going to be difficult to test for and to show, however.

DR. CHRISTIAN: I think one of the things we must keep in mind is that these are screens. And as such, we're doing the best job we can do with our current level of knowledge. It's not really a research project that one is doing when doing the initial screening for potential effects.

But we are totally dependent on the research area for identifying what potential effects we should be looking for. And it's that combination then and development that

will occur with time. So we can't see things as set in stone.

The reason I put out the original studies was, at that time what people were worried about was malformation. Now we're worried about functional alterations. But we don't truly have all of the ways of looking at it yet. We've seen it with immunosuppressants, with immunotoxicants. But if we use those tools for general screening, we may not be sufficiently expert to have relevant information right now. And perhaps some of those things even will not be relevant in the future, but that's the development of research. And we have to consider them. And I think that's part of what we're trying to do here. Should we add it as a part of the general screening pattern? I can tell you, with other compounds that are immunosuppressant we have seen, just as Ralph has seen, effects that don't occur until late postnatal, after puberty. And that's the first they're picked up, with increasing severity.

But it would be impractical for us to do lifetime studies, as well. So what we're trying to do is figure out what can we do on a practical basis in a species that, at least as much as we know, would mimic the human clinical situation

in terms of response to the vaccine, and make sure that there is exposure of the conceptus at some interval that was developmentally similar to the human conceptus.

MR. RENEE [In Audience]: My name is Foulouse Renee [ph], from GSK Biologicals.

Maybe as a feedback to the FDA, the panel, and the audience, I could explain how we design our reproductive toxicity studies at GSK. We do prelim studies, where we test in more than one species immunogenicity. And we select the dose on the basis of the prelim study, as well as the species. Very often, it is the rat.

Then we have for the pivotal study, we have all of the animals which are pre-immunized 30 days before mating, and all of the animals which are immunized only during pregnancy. Now, we immunize besides day minus-30 all animals on day six, 11, 30, 50, of pregnancy. So we try to have the vaccine present during key moments of development of the embryo--fetus.

So we try to maximize the exposure to the formation. And we have good evidence for immune response at these days.

We go for caesarean section at the end of the pregnancy for

half of the animals, and we go for half of the animals to day 21 or day 25 after birth.

Now, after birth we follow the classical parameters, and include also postnatal development, neural development, by assessing the acquirement of the flexes. And we believe that this is maximizing the exposure. We are not looking for optimum levels of individual antibodies. And this has been acceptable everywhere in the world till now.

MR. : I probably missed this, but did you also have at the--what was it?--28 days after birth, did you, besides neurological evaluation, did you have immune function evaluation?

MR. RENEE [In Audience]: What we do is we do the neurological assessment of the pups at day 21. And if there are effects seen, then we can prolong until day 25. Now of course, we follow body weight and other parameters after birth. And we take antibody samples at day four, when we cull the litters to standardize the litters. And we can compare antibody levels at day 21 or day 25. And this is a good indication of exposure to antibodies coming from the mother by the milk. Very often, we find higher

levels at day 21 or day 25 of age than on day five, for instance.

MR. : But you don't do any antigen challenge assay or anything like that?

MR. RENEE [In Audience]: No.

MR. : And do you do any immunohistochemical analysis of the immune-related tissues at that point?

MR. RENEE [In Audience]: No.

MR. : Okay.

PARTICIPANT [In Audience]: Could I ask either the panel or our colleague from SmithKline to frame this question about interval and timing of dosing?

You know, I see two different kinds of vaccines that you might want to do these studies for. One is the sort of vaccine that you might give only one time, like flu or tetanus, during pregnancy; versus something that I'm very concerned about, sexually-transmitted disease vaccines, where you might give vaccines on some schedule like zero-one-six, or zero-one-three-six months.

And there, the individuals who are participating in an IND trial, in addition to being at risk for sexually-transmitted diseases, may also be at risk for pregnancy.

And so there you would be getting a very different kind of vaccine schedule than you would for tetanus or flu. And would that affect this timing and interval of dosing in these repro-tox studies?

DR. BARROW: Yes, I think we would have to design the study accordingly. But we can't actually get away from giving animals a pre-mating vaccination. Because gestation is so short in the animal, we need to give time for the maternal response to develop; which of course wouldn't be the case in the human.

MR. WYAN [In Audience]: Hi. This is Michael Wyan [ph], from 3M Biologics.

We have hundreds of vaccines, both used in humans and veterinary vaccines that have been given to a number of different animal species. And we also have human beings that are exposed to different infectious diseases while they're pregnant, naturally exposed to infectious diseases. My first question is, are we concerned? Are we testing whether or not an immune response has deleterious effects on the fetus? It would seem that we have ample evidence that the normal physiologic immune response is certainly not a toxic reaction.

Now, we heard Mildred Christian say that even in some of these animal studies you could have an irritation on the skin that could result in effects on--I forget what it was, Mildred. Viability of the pups, or whatever. So we know that general systemic reactions, such as inflammatory reaction, could have that kind of effect. But I mean, is that a toxic reaction? So I guess my question is, first, are we testing that?

And secondly, it seemed, based upon some of the things we've heard yesterday, our biggest concern would be an immune response that would cross-react with specific tissues or specific antigens. It might be mimicry, or it might be some other mechanism. So my second question is, are there any examples where we think that fetal antigens would be different than adult antigens, and would pose a different toxic profile to the vaccine?

So I guess part of me thinks that if you could examine a vaccine for tissue cross-reactivity and for safety in tissues from an adult, what's different about the fetus that's going to make this somehow a different problem? Are there any examples of a vaccine that is safe in adults, but is unsafe in children?

DR. BARROW: I'm not saying it's unsafe. In fact, I think the opposite. One example we could use are the group B polysaccharide meningococcal vaccines, where the induced antibody has been shown to target polyciliated molecules, such as neural adhesion cell molecules, which have a different form in the fetus to the adult.

In the fetus, these molecules are polyciliated and are targeted by the antibodies. In the adult, the molecules have been deciliated, and are no longer targeted by those antibodies. So that could give lead to a completely different reaction in the developing animal than in the adult.

PARTICIPANT [In Audience]: But I'd just like to ask Paul with that--and that's correct--has anybody studied that in an animal model in which--Now, here is a great example of cross-reactivity. And we've heard all about all kinds of models for immunotoxicants and for immunosuppressants. But here's a vaccine, and when tested in an animal model, a non-human primate or another model, do the offspring develop any kind of neuronal injury?

DR. BARROW: No, they don't. At least, we've not found any adverse effects so far.

MR. FREES [In Audience]: Lou Frees [ph], ID Biomedical. We've heard a lot of discussion of the need for the conceptus to encounter optimal levels of antibody in the mother, potentially cellular immune responses in the mother; also, to be exposed to vaccine at particular critical time points during development, as opposed to merely the maternal immunologic response.

I think one thing that strikes me as very important, coming back to one of the things that Dr. Christian demonstrated, is that I will readily concede that it is possible, by pounding a pregnant animal with enough doses of vaccine, to achieve an exposure. All those exposures in one treatment group are capable of injuring a pregnant female animal, and thereby her conceptus.

So I would only advocate that we plan these trials very carefully with multiple treatment arms, not every one of which is going to answer every one of these questions.

My second point would be again coming back to Dr. Christian for a moment, and reiterating something I said yesterday. It is not clear to me how doing a trial of an adjuvant only is of necessity for a regulatory package for registration; since the adjuvant only will never be presented to man.

I can see how it's a vital tool to the sponsor in understanding their product. But where the practicality is difficult or the additional manipulations that have to be added to make an adjuvant-alone study possible--many induce toxicities of their own--why is it necessary, or even desirable, to have an adjuvant-alone component to a reproductive toxicity program?

Clearly, if your vaccine demonstrates it, then the onus is on the sponsor to sort out what component of the vaccine is producing it. But if the vaccine, as it will be presented to humans, is benign, what's the additional benefit of an adjuvant-alone package?

DR. CHRISTIAN: What I presented wasn't a full package of an adjuvant alone. Rather, it was a novel adjuvant, which the sponsor wanted to know if it was toxic in and of itself. And what we saw was that it was the adjuvant that was quite irritating and produced the responses in the dam. And as a result, they changed the formulation and got another adjuvant, and went to one that was more standardized.

I think that when there are novel adjuvants, though, particularly in terms of developmental toxicity, it's a

very good idea to study that, just as you would a vehicle or a placebo in a general tox study. Because you want to know if that is affecting the development of the conceptus. And if nothing happens, well, that's fine.

But by having a single arm there at the maximum dose, it sort of gives you a quick way to find out if something should happen at your high dose, whether it is the adjuvant. Although you're quite right--and I think this was your point--that it is in combination possibly different than it is alone, as well.

MR. FREES [In Audience]: Yes, that's right. In combination it may be radically different.

DR. CHRISTIAN: Uh-huh. What we saw with that particular one.

MR. FREES [In Audience]: And that's the important point--

DR. CHRISTIAN: Sure.

MR. FREES [In Audience]: --for the registration of the product--

DR. CHRISTIAN: That's right.

MR. FREES [In Audience]: --for the sponsor. And the reason I bring this up is because, you know, the FDA is extracting opinions here. And the issue that they have to

deal with is registration of the product. I have to deal with knowing what my adjuvant does, and whether or not it's toxic.

DR. CHRISTIAN: It's going back to the old thing: What is the question? And where are you in this stage of development?

MR. FREES [In Audience]: There are two different ones here.

DR. CHRISTIAN: Absolutely.

PARTICIPANT [In Audience]: Could I add something? I just wanted to say that it's not necessarily [inaudible]--

MR. : Use the microphone.

PARTICIPANT [In Audience]: It's not necessarily true that you'll never clinically study the adjuvant alone. Because there's instances in our own company where we have used adjuvant to compare reactogenicity. And so I think including an adjuvant-alone arm in some of your studies--I would be surprised that a sponsor would discover that their adjuvant is irritating in a repro study, though. You know, it seems that there should have been something before that.

DR. CHRISTIAN: Irritating the conceptus.

DR. VAN DER LAAN: I think what Dr. Frees indicated, that if he as a sponsor wants to know what the adjuvant does, it's also for me as a regulator important. And we have no different interests in that respect.

It's a little bit a "chicken-and-egg" problem: What's first? And you as a sponsor want to know, "What is the effect of the adjuvant? What dose should I use in combination with my antigen? And what are the toxic effects of the adjuvant alone, then in relation to the antigen?" And I think those are important questions that cannot be handled only in a combination between an adjuvant and an antigen. You should need, from my perspective, to have also data on the adjuvant alone.

MR. FREES [In Audience]: All good points. I'd only like to add one thing. If I have to physiochemically alter my adjuvant to study it alone, what have we learned?

MR. : Well, yes, that might be a point. But I mean, think back to the list that the lady presented yesterday of the things that ought to be done to test the safety of an adjuvant. You know, there were some interesting "yes's" on there. One of the "yes's" was genotoxicity.

Well, I mean, do you want to do a genotoxicity battery every time you test a vaccine product of an adjuvant? You know, the easiest thing to do is to have the sponsor having done that, and make reference to it.

DR. GRUBER: I had a comment to make. I think we've discussed the issue of adjuvants--given by itself, in its own package, in a [inaudible] master file, combined with the vaccine antigen, and so forth--on yesterday. And I really see that we see all the points.

And regardless of how much trouble I'm going to get into here, I mean, I would like to stress that I think the points made by the audience here are well taken. You have to really ask yourself: What is the best information that we can get to clearly evaluate the safety of the final vaccine formulation? And the type of studies that we do should be driven by that question.

But if I may, I would like to get back to the discussions of reproductive toxicity study designs, per se, and animal models. I am struggling with how to really tease out and look at potential developmental toxicity that may be induced by potential intrinsic toxicities of the vaccine

antigen or other components in the vaccine formulations; versus the immune response.

And what I think, what I have been hearing this morning and this afternoon, is that looking at the potential for--let's call it immunopathologic effects, for lack of a better term--doing the studies that we have been suggesting them to do in the guidance document, is probably not going to be feasible, given the differences in immune system maturation in the animal species that we have available to us for these types of studies, compared to the immune system and its maturation in humans.

So what do we have to do? Should we restrict developmental toxicity study designs to segment two studies, or extended segment two studies, in order to just answer effects on organogenesis or fetal development? Or should we extend the studies? Or should we do additional studies to evaluate post-weaning assessments, if this is what needs to be done to look at potential effects on the immune system of the offspring?

And right now, I'm really struggling with if we really should require that as a sort of one-packet approach, or if we should consider what was also mentioned by industry when

we got the comments to the document. Should we consider a tiered approach, sort of looking at the developmental tox study as a signal-generating assay? And if we don't see any signals, we still can't say for sure, of course, that "My product is going to be safe when given to a pregnant woman."

Or should I go ahead and also look at immune response evaluations by doing additional studies? And that's something that I would like to have discussed, not only by the panel members, but I would also like to hear how the audience and industry feel about this approach.

DR. CHRISTIAN: Yes. If I can, let me just give you one thing that might be your first tier. And that would be, in the same species, compare the immune response in a non-pregnant and a pregnant animal, and see if there is a difference there.

And then, on a tiered approach, look at what we would usually look at in the parameters we can recognize. Because even with first trimester insult in a rat or rodent model, we had the progenitor cells. Even if it isn't fully developed.

And you're going to get certainly the typical responses, with the exception of function, by C-section and certainly by postnatal day 21. You're going to see it as by ability effects and weight gain effects. They'll be noticeable. If you want to add in some function, fine.

But that whole field is evolving from "behavior," which 20 years ago we finally got changed to "function." But now "function" isn't fully defined. And so that's an evolving area that will change with research.

What I would think is the first tier, if they had exposure during gestation and were allowed to go to weaning. That should certainly give you a pretty good initial tier one screen in one responsive species. And I would just suggest that as a good place to start. Then, if you see effects, you go to the next levels.

DR. GRUBER: Thank you.

MR. RUSSO [In Audience]: [Inaudible] Russo [ph], from Merck.

I think that the discussion was perhaps too focused on immune-mediated toxicity, to the extent that we struggle as it is to develop animal models that would be suitable for

even assessing the efficacy of new vaccines that we are developing. And to really focus on immune toxicity--

[Tape Change.]

4A MR. RUSSO [In Audience]: --the way to do is to really assess whether there is this thing out there, and then trying to figure out how to do it. We have no discussion whatsoever here in a relevant model for cell-mediated immune responses. Most of the discussion was on antibody-mediated toxicity.

There was some presentation in terms of cell-mediated immune responses, but nobody discusses the host of genetic-controlled immune responses, even in animal models where parasites may skew the responses according to TH2 types, and so forth and so on.

DR. GRUBER: Yes, you are absolutely right. Just I wanted to answer this. I guess we are all a little bit uneasy to say that, okay, we're focusing, if you look at immune responses, at antibody response. But the reason why this is, is because we perhaps have the best assays validated and reproducible as is to look at antibody responses.

If you start discussing cell-mediated immune response, the question is: Where do you want to start, and where do you

want tc end? And do you want to throw in the cytokines profiles that you could potentially anticipate?

I guess I know that that is something that needs to be addressed in the guidance. I feel, though, that perhaps we should give some thought regarding how much and what to assess in terms of immune response. Should it go beyond antibody evaluations? Should it perhaps be driven by pilot or preliminary studies, to see what vaccine antigen do I have? What type of immune response do I expect for it to elicit?

Is it going to be more--Sometimes the adjuvants that I added will sort of shift the immune response from a TH1 to TH2. And so that you may want to say, "Okay, I'm going to look at certain cytokines, or certain cell-mediated responses."

But I'm not quite sure if we should build in as a first evaluation sort of a full assessment of the potential immune response repertoire. Because it could again lead us to data that may be very hard to interpret, at least in 2002.

I mean, I'd like to hear more comments. If you feel it's necessary to evaluate more than antibody responses, you

know, we would like to hear this. But I think we're going to get--You had a question, a comment to make?

DR. HASTINGS: Well, Marion, can I follow up? This question is directed to Mercedes. For the therapeutic cytokines, have you got reproductive toxicology data for those?

DR. SERABIAN: Do we have it?

DR. HASTINGS: Yes.

DR. SERABIAN: More and more, yes, we do. We are generating data--mainly, seg two studies, teratology studies--with the cytokines. Again, it depends. The big thing there is antibody development, and basically clearance of the material. So it's basically not effective.

But what's your--Ken, was that your question?

DR. HASTINGS: Well, just to get at that. Because you know we were talking about other immune-induced molecules that might eventually be manifested as like teratogies.

DR. SERABIAN: Right. No, you do see some of them--Well, there are teratogenic effects with some of them, yes.

PARTICIPANT [In Audience]: Well, I'm struggling a little bit in the back here, so forgive me. This may be in part my naivete of this whole area.

But this is an extension of the gentleman over here. And he said when you talked about your tiered approach of looking at the toxicology or repro-tox assessments, can you, instead of looking at just gross or these larger changes that you're looking through, through the entire cycle of reproduction, connect it more to what is epidemiologically relevant?

I know the gentleman from EPA made some connections between effects that he saw in animal models and studies in Inuits, etcetera. I know that there are studies out there looking at immunotoxicological effects caused by the immune system in different ways when it's stimulated in different ways. And maybe one of the panelists could enlighten me on some assays that we could use, in this sort of area of immunotox, to really make a connection between the pathology or the function that we want to look for, and some sort of epidemiological feature that is a problem out in the population?

It seems to me like, if we're just looking at just any effect, as the gentleman said yesterday, you can create an assay just to create an effect. The issue is whether it's relevant or not.

DR. SERABIAN: I just want to, on top of that--I agree. I guess to me, when you mention this about tier, that sounds great, that's important; but again, what's the signal that you're looking for, and how appropriate is it, as to what testing you're doing?

And I guess, just kind of an editorial, when you say "immunotox" I kind of cringe. I think maybe you mean a module [inaudible]. I don't know. It's not always immunotox that you're looking for. It could be immunosuppressive. It could be immunostimulatory. And the word "tox" kind of--At least personally, I don't care for that. Okay.

Ken, do you have any suggestions as to the testing maybe?

DR. HASTINGS: Well, clinical immunotoxicology is a very poorly developed field. And there aren't that many things--I mean, you know, I guess the most important thing you would think about doing is just the prospective cohort kind of study, where you would look to see in the children, do

they go on to develop susceptibilities to certain autoimmune diseases or things like that.

I mean, that's what happened, I believe, with cyclasporin. You find that there is a higher incidence of autoimmune disease in babies born to women who were taking cyclasporin. So I think mainly that's the kind of gross epidemiologic studies that you're kind of stuck with.

PARTICIPANT [In Audience]: I just wanted to make a comment. Marion, I think you framed the question really well. I've been a little confused by the discussion. There seems to be confusion between the immune system as the agent causing the toxicity, with the immune system as being an end point in the fetus for the toxicological effects of the vaccine. And I think you really need to separate those two issues in this discussion.

Yesterday, in the general tox studies, I think at least most of the consensus seemed to be that the general measures of toxicity were sufficient, and that special immunotoxicity tests as end points were not necessarily necessary. And I think that it is also true for our developmental toxicity tests.

Some of the talks this morning were very nice descriptions of the development of the immune system. And at the end of Dr. Barrow's test, he focused on the development of the immune system as to the timing of doses. But as toxicological end points, as the end points of these immune-mediated toxicities, we're not only worried about the development of the immune system, or development of the CNS, or any major organ system.

So I really think, as far as talking about end points, the emphasis should really be shifted away from the immunological system, and focused more generally.

DR. BARROW: I think the point was when we're dealing with vaccines, immunological endpoints, or in addition to all the other parameters we normally look at for other therapeutic areas,

PARTICIPANT [In Audience]: But I would ask why. Do you do that for drugs?

DR. BARROW: Yes, we do that for drugs. If we're testing a CNS-active compound, for instance, we pay particular attention to CNS development.

DR. SERABIAN: Okay, just real quick, I think that's going to be, hopefully, a focus of the next hour, or the end points. So we can continue with that.

PARTICIPANT [In Audience]: Sorry, one more question. I debated a lot whether to pitch this out here, but I'll just throw it just to see what happens.

The whole discussion about the potential issues related to vaccines and whether they cause a toxic effect to the animal model, to the patient, kind of leads to an interesting quandary. Some folks who work in vaccines feel that creating an immune response sometimes causes what some people would call a toxic effect; i.e., a swelling, redness, pain, sickness.

In some vaccine strategies, it may be a good idea to make a person a little sick initially, so that in the end they're protected from the infectious agents that actually may cause death or severe sickness or severe disease.

I wonder if by creating parameters like this we create vaccine strategies that won't impact a person's daily life, and won't make them sick, won't make them feel any pain; but may not in the end be as effective a vaccine as we could possibly create.

DR. VAN DER LAAN: I think you're fully right, that developing or introducing a vaccine in an animal will lead to an immune response, and the immune response is a physiological one that leads to a lot of disturbances that we have discussed yesterday, too. We indicated that, also. The characterization of the immune response is more important than the definition or than defining or evaluating whether or not that response is leading to immune suppression or other things. The purpose of your evaluating the immune response is important.

With respect to the developmental aspects of giving a vaccine during pregnancy, it is important that introducing a vaccine may lead to an adverse effect. And your first effect is, of course, vaccination in the pregnant animal. But then it may also lead to an adverse effect on the fetus. And that's the problem that we are dealing with. And is the adverse effect on the fetus a direct abortion, or a malformation, or a functional malformation?

DR. CHRISTIAN: Yes. To carry that on, I think that the other thing that we have to have these types of studies for is ultimately in labeling. A woman is inadvertently vaccinated during pregnancy, and her question to her doctor

is, "What should I do now?" And so we need to have some kind of indication.

If there are no adverse effects seen in these types of studies, at least the doctor can say, "We don't think it will be a problem." If we know that the response was such that the embryos died, then we can say, "Well, at such-and-such a multiple, we know that this occurred," also.

So remember that the adverse effect, even if it is a normal physiological response, can be a pharmacotoxic effect for the conceptus. And that's always the two sides of the concern.

PARTICIPANT [In Audience]: I guess what I wanted to just mention was--and it follows on nicely from that--I'm no immunologist, but it's my understanding that often in the first trimester, due to hormonal influences, women's ability to mount a cytotoxic T cell response is somewhat subdued. And we see that in terms of evidence of infection with toxoplasmic [inaudible], and other sorts of parasitic and viral agents like that.

So when it comes to developing a vaccine where we want to generate a robust CTL response, and a woman is inadvertently vaccinated in her first trimester, I think we

would want to know, is there some model so that we could understand what would happen to the fetus? You know, is it going to cause an abortion? Because there must be some reason why we have a subdued CTL response in that first trimester.

And then I'll tell you, the other thing that really causes me concern is that we do these studies, and we do put something in the label to give the physicians some guidance. But we actually don't understand the influence of confounders, like women smoking through pregnancy and things like that. And then what does that mean, in terms of us getting sued because we've got something in our label?

And you know, we've done these lovely experiments in a controlled environment, and sought some understanding, and we're trying to provide some guidance. But it's also a very scary thing to sort of embrace, as well.

DR. GRUBER: Oh, yes. Yes. Yes. I guess nobody can argue with logic.

[Laughter.]

DR. GRUBER: But I think there's one thing that I wanted to actually throw out here. And that is, we do developmental

toxicity studies for preventive vaccines perhaps in an attempt to be able to possibly identify potential developmental hazard, or using these studies as a signal-generating tool. I don't think that if we put the data into the label, that that is equal with saying, "Now we're going to make a prediction to human risk." Because everybody does understand that there is a difference between man and beast.

I think that, however, not having the data is really something that Francois discussed yesterday morning: It is sticking your head into the sand. And I think having some data is better than having no data at all. But I think that the difference between really predicting human risk, and using these as signal-generating tools, I think is an important difference that we need to keep in mind.

What I wanted to actually do before we break for coffee is--And I know this is not going to be a five-minute thing. But we've put a question up there. And perhaps in the discussions that Paul Barrow had we've already sort of answered these questions a little bit, and we discussed it a lot yesterday. But I think this is something that we should briefly turn our attention to.

And that is the question--Perhaps it's best framed again in: My animal model that I choose should be perhaps driven by the kinds of questions that I want to answer. And if it's really that what we're going to do here is a first tier evaluation, where I'm going to do a developmental toxicity study, and I carry this out to birth or weaning of the animal models, then perhaps I'm going to choose my animal model accordingly. And if I want to look at immunotoxic, immunomodulatory effects, I may have to look at another animal model, or do an additional study.

But how do we feel about the question about a relevant animal model? Can we define it by, as we naively stated in the guidance document, the ability of the species to mount an immune response? Or do we have to be a little bit more precise in our definition? And what other parameters do we need to consider?

[No Response.]

DR. GRUBER: Anybody?

DR. CHRISTIAN: Well, it certainly should get across the placenta, also. So you need both an immune response and crossing the placenta. Having only immune response, and it doesn't cross the placenta, it doesn't answer your

question. Getting across the placenta without an immune response doesn't do it, either. So if you had none that did both, then certainly one of those would be better than none. But ideally, it should be both.

PARTICIPANT [In Audience]: I would only like to iterate a point that I made yesterday. It is that in this definition it's great, I think it's probably--The issue I have is, if you have an animal model, whatever model you select, that if you're using a vaccine modality that promotes an immune response in that species, you're probably okay.

If you're using a vaccine modality that doesn't promote an immune response in that species and is a very human-specific pathogen or vaccine modality, then you may be stuck. You know, you won't be able to address any kind of immuno-issues that are created by the vaccine. You may not be able to address some of these reproductive toxicology issues related to immune response.

You know, it's a real tough issue, because as we get more creative with our vaccine strategies, animal models may or may not become more relevant.

MS. MURSA [In Audience]: I'm Sandy Mursa [ph].

I guess I'm really confused. Because it seems to me that if what you're looking for is whether IgG crosses the placenta or not, then Dr. Barrow has outlined a nice way to figure that out. And you can determine that your animal model is probably a rabbit, and we don't have to carry on this discussion for very much longer.

But if that's not the thing, because it's hard for me to visualize--and I'm not an immunologist--but how simply IgG crossing and being available to a fetus is going to cause a malformation. That's pretty hard for me to understand. So I think that's probably not the point.

And then, you know, you say, "Well, if it cross the placenta," but I don't know what "it" is. You know, is "it" the antigen? Is "it" IgG? Is it--I'm not sure.

I don't think it's as simple as this. And if it is as simple as this, then I think you can just take and say a vaccine that mounts an antibody response against virus "X"; look for IgG; and then you never have to test another vaccine for virus "X" again. It doesn't matter what the construct is. It doesn't matter anything, if IgG is the only thing we care about.

DR. VAN DER LAAN: Let's try to go further in thinking. Yesterday I have indicated that in Europe we have thought about a relevant animal model as an animal model in which you can induce a change. But that's not always possible. And in this case, we have to use a case-by-case approach. Maybe if you have a polysaccharide vaccine, then the IgG response and the IgG transfer through the placenta might be very important. If you have a live attenuated vaccine, then the placental transfer of the virus is important. Or, as for small pox, it's thought to be that the interferon response might lead to an abortion very early in pregnancy. So it might be you should also in this respect use a case-by-case approach.

And then the criteria for what is a relevant model are much more derived from a comparison between the immunological response in humans to the infectious agent or the vaccine itself, compared to the animal model. I think that all those points have to be considered in this respect.

DR. LAMBERT: I would like to push the idea a little bit further. If we would like to develop a decavalent vaccine, and we should not be able to demonstrate immunogenicity in any species for the ten antigens, what should we do?

Should that be a good excuse for not doing a study? Should we use a species where we have a maximum of immune responses?

[No Response.]

DR. VAN DER LAAN: We are all quiet. We have no answer. I think that's the most difficult situation, and it's very difficult to handle. But maybe there are people in the audience that would indicate, "Okay, animal studies are not necessary. You can directly go into man." I think that nobody has that opinion. But we have to struggle with that. I have no definite answer. It depends on the case.

DR. SERABIAN: Again, look behind you. I think that's one of the--Yes.

Any more comments with respect to the question I have up here now? No? Thoughts? Okay.

PARTICIPANT [In Audience]: Obviously, I'm working in an area where this is directly relevant, so--And the gentleman from Merck yesterday sort of helped answer this. You know, it's sort of a double-edged sword. You can switch to the animal system where--You know, like taking animal antigens or taking an animal-suited or a model-suited virus. Let's just take a virus, for instance, as a good example.

Let's say you're working with a human pathogen and there is an animal model, but it's not the human pathogen; it's an animal-adapted pathogen. You can use that animal-adapted pathogen, but you have to recognize that there's going to be big differences between the two, because it's an animal model and it's not going to be a perfect model.

But the challenge is, from a vaccine production point of view, that you have to develop these two things together, in the same manner, in the same way, to really adequately test the different relevant toxicological issues that may be related to these things.

And God forbid that the pathology or the pathogenic features of the animal model differ in any way from what happens in the human. Then you know, it's another whole issue to deal with.

So I don't know, it's sort of a very, very difficult problem. And I would love to hear folks who may have more experience in this give some sort of advice, because I think there's probably quite a few people in here who will be faced with similar problems related to this.

DR. VERDIER: I would just like to give one remark regarding the question behind me. I think it's really

difficult to answer to this question without more detail about the specific vaccine. Because you have to consider the human data. Do we know something about the same infection in humans?

We have also to consider the nature of the vaccine. Is it a live viral vaccine? And in this case, the risk can be higher compared to a recombinant protein, for example. Do we have a strong adjuvant which can trigger a different production, or do we have no adjuvant at all?

So I think when we will write the non-clinical safety package in the IND or in the pre-IND, I think we have to take into account all of this information; and particularly, information regarding infection in humans. Do we have data which indicate that the pathogen can trigger abortion or can trigger cytokine release which can lead to abortion?

We were discussing with my neighbor about the different potential strategies according to the nature of the vaccine. And I think that if you deal with a live viral vaccine, it's very different compared to a recombinant protein. And we have to take that into account. We cannot

answer "Yes" or "No" to a question. We have to take globally all of the information available.

DR. SERABIAN: I think that's an appropriate time to break. It's about 3:15. How long do you want to go? Till 3:45, then. Then we'll come back.

[Recess.]

DR. SERABIAN: Okay. Basically, I'd like to introduce the two remaining people on the panel here that have not been more formally introduced.

Dr. Verdier, I don't think I need to read his introduction, since you know him quite well from yesterday and today. And he will give a very small presentation--two or three slides, I think--to just start us off.

And Dr. van der Laan, he is a pharmacologist and toxicologist. Since 1990 he is head of the preclinical assessment group of the Medicines Evaluation Board of The Netherlands. It's located at the National Institute for Public Health and the Environment. In this function, he is responsible for giving advice on preclinical safety aspects for The Netherlands College.

On behalf of the Medicines Evaluation Board in The Netherlands, he is a member of the Safety Working Party,

the SWP, of the CPMP. In the SWP, he was responsible as a rapporteur for the note for guidance on preclinical, pharmacological, and toxicological testing of vaccines, as well as on the revision of the note for guidance on repeated-dose toxicity, which included immunotoxicity aspects.

IMMUNOLOGICAL ENDPOINTS

PRESENTER: FRANCOIS VERDIER, PHARM.D., PH.D.

PRODUCT SAFETY ASSESSMENT, AVENTIS PASTEUR

DR. VERDIER: Thank you, Mercedes. I will just briefly introduce the subject about what are immunological end points. And I think we have to ask the following questions.

With the immunological end points, we want to confirm the relevance of the animal model. And we were speaking about surrogate markers, antibodies in the mother or in the fetus. An antibody measurement can be used as surrogate marker to confirm that the animal model is partially relevant.

We can use also immunological end points to evaluate potential adverse effects. And we will see when and how.

These immunological end points can concern the mother, the fetuses, or the pups, on any other model.

To illustrate these immunological end points, I would like just to show you this graph which represents the cytokine balance during the pregnancy. And it's true that if you interfere with the cytokine equilibrium, you may induce pregnancy loss. So you can imagine that if you give a live virus and if you trigger high production of interferon, you can perhaps impair the pregnancy.

So about immunological end points, I don't know if we should measure the cytokines, but at least we can imagine that if we give a strong stimulus, if we give a live virus which will really trigger a strong cytokine change, you may have changes in the pregnancy.

Regarding surrogate markers, I think when we are measuring IgG we are not measuring IgG for the potential toxic effect. We are measuring IgG to show that we are triggering something to show that we have selected an animal model which answers to the vaccine.

And I have just reproduced here what we are doing. And Paul presented this kind of treatment design in his presentation. In fact, we are immunizing the animal before