

Panel Discussion

DR. ZEISE: Maybe if we could move to the panel discussion. Kim, you'll be the first questioner there. So if the rest of the panel could please come up? We have Dr. Dale Hattis also joining us, who you will hear very shortly give a full presentation on pharmacokinetics. He is a research professor at Clark University working on issues of risk assessment and risk policy.

DR. PORTIER: Before you get started, Kim if I could respond to some of the previous questions a bit? I just want to make a brief comment.

The relationship between reproductive hazard and cancer could be easily assessed with the data in the NTP archives. The NTP has done a number of reproductive, developmental reproductive toxicology tests in Bob Chapin's lab with a number of chemicals for which we have also done the cancer bioassay.

You are not looking at the same animals; you are not looking at the reproductive effect in the same animals that you are then looking at the cancer effect, but you could certainly infer some correlation from that type of data, and I would look at that.

And, Walter, the only data I am aware of on an infectious model in an animal that has been looked at for carcinogenicity is a hepatitis model in the tree shrew. But that is a slightly different issue than the one you are looking at here. It is the toxicity from the hepatitis in the tree shrew, followed by aflatoxin exposure. That is the only one I am aware of.

DR. HOOPER: A question for Chris and Lucy: time periods of susceptibility seems to me a crucial issue, like exposure at different times gives you different susceptibilities to cancer. Can you think of, or what do you have in mind in terms of animal study designs, which would get at that issue and which are feasible and which are not?

DR. ANDERSON: Well, obviously you can control when during development you expose an animal, which is how these various correlation studies have been conducted. And one could simply do more of those.

Pulse exposure will work for everything except for bioretained chemicals, which do not tend to be good perinatal carcinogens anyhow in the rodent. One could examine animals pulse-exposed at different stages and then look at the cancer end point.

DR. ESKENAZI: I guess along those lines we have seen over the past day and a half many examples where postnatally the rodent differs from the human. I wonder if you could also speak to the relevance of certain kinds of tests of looking prenatally in the rodent and believing that that corresponds directly to the human, and if there are better models that could be used for looking at prenatal exposures in humans.

DR. ANDERSON: Obviously the non-human primate is a better model for the human than is the rodent, and we actually worked on that for a number of years. Monkeys are susceptible to transplacental carcinogenesis, at least to ENU. The monkey fetus has characteristics that are more similar to those of the human fetus than the rodent. I think the differences between the rodent and the human are fairly obvious. I am not sure what you are looking for beyond that.

DR. ESKENAZI: I think one of the things that we are grappling with also is quantitatively how do we address that issue. Let us say that we do have some postnatal data. Should we be using it for prenatal, and could we maybe use pharmacokinetic models and so forth to look to try to address that issue quantitatively? I don't know, Dale or Chris, if you can comment on that?

DR. PORTIER: I think the problem is it is chemical-specific and it is animal-specific; it is a very difficult question to generalize. I think if I were looking at the issue, as you have just posed it, in terms of having information on postnatal exposure and a certain end point or outcome, I would approach it first by trying to develop a general developmental model that describes the various stages and their importance in the development of the

outcome I am looking for. I would try to take the data I have on the postnatal exposure and make that work within the context of that model. Then I might feel confident in generating the hypothesis that I can back up into the prenatal area and develop a design that would address the prenatal potential directly from the model. But I would be very wary of the extrapolation backwards in time from the model to make that type of statement.

I think part of the problem is a lot of what we heard in the last few days, no one has actually sat down and tried to develop some sort of quantitative structure which describes the general natural process. Given that type of structure there is a lot we can do to address these questions of extrapolation in time and extrapolation in age, and we just don't have those types of quantitative structures to work on.

The rat estrus cycle is one clear example where the literature on it is almost barren, and yet you would think somebody could have sat down and developed a model for the rat estrus cycle and described exactly quantitatively what they think is going on.

DR. HATTIS: Yes, I want to reinforce that we need the basic quantitative description of the growth and the relevant tumor cell types that are likely to be susceptible to carcinogenesis. I mean, how many cells are reproducing when and over the different developmental periods in both humans and animals. Now with some of the more, modern molecular biological techniques there is every hope that we are going to be able to figure out what the transition rates to the relevant cancer are, that is, the molecular pathological transitions on the way to cancer.

If we can count procarcinogenic mutations in ordinary somatic tissues then we can build the model much more solidly as to what's happening at different ages. Also, we can directly test how many of those mutations do you get per tissue. Without doing a two-year bioassay and waiting for the tumors to develop we can figure out, how many of these relevant mutations do we get in relevant cells as a function of dose, soon after they actually happen, and we can then directly measure that sort of process.

So I think that in this coming century, maybe even the early decades of the century, we are going to have vastly improved information to address these questions.

DR. GOLUB: I am really following up on Lauren and Kim's comment, which has to do with the prenatal/postnatal difference. It is very discouraging that animal models just have no capability of modeling a third trimester human exposure where the mother is exposed. The fetus has metabolizing enzymes, is producing a lot of cells, a lot of cell proliferation and differentiation is taking place, and the animal models do not address that.

Now you mentioned non-human primates. I wonder if any thought had ever been given to guinea pigs or ferrets, or some other animal that has a third trimester where you might be able to model that developmental stage better.

DR. ANDERSON: There have been very few such studies -- a rabbit has been used to an extent, but you still have a much shorter gestation time, a much more immature fetus at birth, and a different structure of the placenta. Guinea pigs have probably been the least studied of all of the rodent species with regards to transplacental carcinogenesis. I am not sure why that is.

DR. GOLUB: My second comment would be sort of along the line of what Chris said and what you said. It is really true that the developmental people do not look at the transplacental cancer literature and the cancer people do not look at the transplacental cancer literature. You have been very effective in disseminating that. I know you have passed along a lot of literature to me just through your network. But, I wonder if you have thought about having an on-line database of the transplacental cancer studies so that the people could find it all in one place and maybe get to use it a little bit more in risk assessment.

DR. ANDERSON: You think there would be a call for that?

DR. GOLUB: I think the easier it is the more likely it is that people will use it. It is difficult to find that literature; for example, there are a lot of European studies. It is not

like the NTP literature where you can sort of sort through it and find it, so I think it would be valuable. Yes.

DR. ANDERSON: Well, this is something we could discuss.

DR. BARONE: Dr. Portier, I have sort of the follow-up question on one of the slides in your talk. You presented this matrix model of cell signaling in proliferation as it relates to cancer, I assume. Has that model been proved, tested with data for development, since many of the same signaling pathways are, of course, critical for development?

Also, given what you just said about pharmacodynamic modeling and the importance of pharmacodynamic modeling, in addition to the PK modeling, it seems like that is a real critical niche that needs to be explored if we are going to start dealing with critical windows of exposure and how that might predict adverse effects.

DR. PORTIER: That model was for cell cycle in an individual cell, so it is not a tissue issue and it is not an autocrine signaling issue. None of that is covered in that type of modeling. Most of it is developed based on in vitro data, not in vivo data. One of the benefits of, or potential benefits of the use of technology for studying mRNA, which is not just the gene chips, and I agree with you, those may not get us anywhere, but Tackman and other techniques that are quantitative and quite well-established. One of the benefits of those is you will be able to study this in vivo, in populations of cells in vivo.

We have not done any of that for the developing fetus. The knowledge that is available on the signaling pathways during development are clearly not up-to-par. We barely understand that in a developed adult animal. I think that is a critical area, a critical research need, and I see some of this technology as being extremely useful in pushing and filling gaps in that area.

DR. BARONE: You also mentioned epigenetic events in your talk. Given sort of the discussion of genomes, it seems to me that it would be important to look at epigenetic

events as well and how you incorporate that into your models. I would like to hear what good ideas you have.

DR. PORTIER: Well; now you are at the cutting edge of what causes cancer. There are a number of theories about how epigenetic events can actually lead to a carcinogenic finding without a single change in base pair sequence. We have actually developed a single model for that, a very simple model, and it is in the literature. I think it was in the journal *Mathematical Biosciences*, which I am sure all of you read religiously.

Anyway, it is not an area of clear understanding. One of the nice things about having sat down and developed that model that we used in that situation was it forced us to try to think about what this theory means quantitatively. I think we got it entirely wrong, but at least it was functionally usable for us.

I think it is an area also of development. I do not see any obvious near-term solutions on how epigenetic, permanent modifications in cellular function can be used in understanding the carcinogenic process until we can actually identify them fairly readily. I do not think we are there yet.

DR. GINSBERG: Gary Ginsberg, Connecticut. As many risk assessors in this room know, a standard way to estimate the risk from less-than-lifetime exposure is to prorate the dose to the carcinogen by a full 70-year lifetime window. Because the assumption is that the animals were exposed for "lifetime" duration. We all know that most animal bioassays start at five or six weeks of age, sexually-mature animals.

So your data, Chris, on having a different averaging time is very interesting in terms of equating back to chronic exposure from these stop/start experiments. But I am aware for vinyl chloride, for example, that the EPA has basically a no-proration policy regarding young children's exposure because of some of the data that -- in animals, that where a couple of weeks of exposure early in life -- I think between day eight and day 23 for example with vinyl chloride -- got the same tumor response as starting later, but going for two years, the same basic hemangiosarcoma response.

In that case we have an initiating carcinogen for which the risk assessment is completely different than if one assumed 70 years was the proper baseline denominator for the dose. Now we have got no proration.

Are there other chemicals, other situations, or should we just consider anything that's an initiative carcinogen to be in that same category? What kinds of data are out there? And can any of the single-dose, you know, newborn mouse model data be used along similar lines?

DR. PORTIER: Good questions. The studies done by Dedrick on the chemotherapeutics looked at the relationship between the rodent dosage and the rodent studies and the human dosage and the clinical intervention for the cancers and addressed the question of what dose-metric seems to make sense and how to average the dose. I would suggest you look at that paper.

David Rall did a paper about 15 years ago looking at this same issue. Surprisingly, the two groups concluded pretty much the same thing. I would suggest you look at that as well.

In my experience in looking at all the chemicals I have looked at -- for which I have had sufficient human data to do dose-response analysis so that I could address the question (because you've got to have both the human data and the animal data in such a way that you can address the dose-averaging issue) -- for dioxins we found that dose-averaging was not bad. The animal cancer response and the human cancer response was sort of in the same range if you dose-averaged by body burden, not by daily intake.

For aflatoxins we found that daily intake was the better measure. If you averaged daily intake over a lifetime you got approximately the same response in the animals and the humans. So, it just varies in the data you have.

DR. GINSBERG: I think the key question is were those aflatoxin studies done in a newborn situation and then the dosage stopped, versus just in sexually mature animals to see the comparative potency?

DR. BARONE: No. The human studies clearly included some primary liver cancer in children, but the animal studies were all adult animal studies.

DR. HATTIS: I wonder if this is a good place for me to step in and make a comment or two on Chris' excellent analysis. I think that you have got a very good start to analyzing those data. I think that there are a couple of other steps you should consider. And one is, in particular, the trying to segregate out possible pharmacokinetic effects.

DR. PORTIER: We did that, I just did not have time to present it.

DR. HATTIS: Oh, okay.

DR. PORTIER: There was no apparent association with half-life, no apparent association with initiator or promoter or genotoxicity of the agent, and no apparent association with body weights in the animals. We looked at a lot of those issues, and if I would have seen anything that was an apparent explainer of the averaging I would have given it to you.

DR. HATTIS: But it is important that the pharmacokinetic non-linearities be ruled out, because you are giving high-dose exposures.

DR. PORTIER: I am not the only fellow in the back room that will ask that question.

DR. HATTIS: It matters to the actual practical use of the results. I mean, you have a solution for the general case where you do not have this excellent information, that presumes that the same averaging time rule would be likely to be followed at low doses, and low-dose, much lower dose rates that you would want to be assessing risks for in the human population. That is quite reasonable if, in fact, the mechanism is a pharmacodynamic-type mechanism where exposure early on in the life essentially causes the same number of tumors and the exposure you get later on in life essentially is irrelevant.

If the explanation were to have been that you saturated a DNA repair capacity or you saturated a detoxification capacity then that same expectation would not hold. So, I think it is important that you do rule out that. Also the other kind of evidence that you could use to check on your result, to check on this pharmacodynamic type of explanation, is in

fact the time pattern of the tumor developments. I know you must have that in your database.

DR. PORTIER: No. These are occult tumors. These are tumors detected at the time we sacrifice the animals. With the exception of a mammary tumor or the skin, tumors you just would not have that information.

DR. HATTIS: Yes, all right. So the time pattern of the development of tumors would be different for the two kinds of explanations. And, of course, if you do not have the information, you do not have the information.

DR. PORTIER: About the pharmacological differences, let me explain exactly what we did when I said it did not differ by half-life -- because obviously we did not have the half-life for all of these chemicals. What we did was calculate the oil/water partition coefficients for the individual chemicals and assumed that half-lives would, to some degree, follow the oil/water partition coefficients. We found absolutely no linkage between them.

The problem is, you are right, a lot of these things should be included in these types of analyses, but you are talking about a much bigger problem again. Now it is getting back to the whole database, and looking at the whole issue.

DR. HATTIS: It is not just a matter of pharmacokinetic half-life. It would be helpful to have, in fact, some assessment of the saturation issues.

DR. PORTIER: But that requires a tremendous amount of information, more than probably is available on these compounds. There are ways to look around it; I can get you a copy of the paper.

DR. HATTIS: Yes, I know you have data, pharmacokinetic data for butadiene, which is one of the compounds, but there probably are few others that you have. And you've already had the investment of doing a two-year bioassay for these, so probably the incremental investment to do some basic pharmacokinetics is not so terrible.

DR. PORTIER: It roughly comes out to a quarter of a million dollars per chemical to do a full pharmacokinetic workup on each compound, and each sex and each species.

DR. ZEISE: Maybe this could be the final comment or question.

MALE VOICE: A year ago I was a project manager for the company called the Kevric Company in Maryland, that was charged with the responsibility of beginning a database for the entire National Cancer Institute to classify all cancer research funded by the cancer institute into 34 different categories. It will be searchable by a slew of different key words and classified according to basic science, treatment, prevention, modeling, animal modeling, human modeling, all sorts of different ways. This has been done for 8,000 abstracts in the last two years and it's going to become an ongoing database that'll be added to each year.

That exists for intramural cancer research and it's being done for extramural also. It is a mandate by Rick Klausner, Director of NCI. I have the name of the project officer at NCI, but I do not think it is out yet. They are formalizing the database, but it is going to be available over the web for anybody to look up anything they want on any kind of cancer research being funded by NCI. They could probably use input as to more fields to make it more useful to people in fields other than carcinogenicity.

DR. PORTIER: I personally would like to see it go a step further. One issue we are pushing from within NIEHS is that if you do research funded by the U.S. government in the area of toxicology, not only do we want to know about the published research on it but we want the raw data after you publish, and archive and database that information so that it is available for use by other people -- not just your summary analysis of your own information, but the actual raw information itself.

I think that would be a tremendous resource to avoid redundancy, to get better clarity in the analysis. I do not see a reason, other than in the area of epidemiology that is a clear problem, but I think in the area of animal testing it should not be a problem.

MALE VOICE: Let us start with the data that are funded by the government. These data belong to the people and, the researcher should get his or her whack at analyzing it and publishing it, and then I think it should be made public. My experience is generally when you ask an experimental biologist for the data you get the data; when you ask an epidemiologist for the data you generally do not get the data, except if he works for the government directly and then you do get the data.

DR. ZEISE: I'd like to thank the speakers and the panel participants. (Applause.)