



SOT FDA Colloquia on Emerging Toxicological Science: Challenges in Food and Ingredient Safety

**February 20, 2019—Redesigning the Rodent
Bioassay for the 21st Century**

US FDA, Wiley Auditorium, College Park, Maryland • Live Webcast

Real-Time Captioning

Note: this is not a transcript.

February 20, 2019

Redesigning the Rodent Bioassay for the 21st Century

Chair: Suzanne Fitzpatrick, US FDA, College Park, MD

Co-Chair: Warren M. Casey, NTP/NIEHS, Research Triangle Park, NC

- 8:30 AM–8:45 AM **US FDA Welcome and Overview**
Admiral Denise Hinton, Chief Scientist, US FDA, College Park, MD
- Welcome from SOT and Introductions**
Suzanne Fitzpatrick, US FDA, College Park, MD
- 8:45 AM–9:25 AM **The Chronic Cancer Bioassay Is Frequently Conducted for Pesticides When It Is Not Always Needed to Protect Human Health**
Doug Wolf, Syngenta Crop Protection Inc., Research Triangle Park, NC
- 9:25 AM–10:05 AM **Threshold-Based Risk Assessment Is the Same for Cancer and Non-cancer Endpoints for Non-DNA Reactive Carcinogens**
Samuel Monroe Cohen, University of Nebraska Medical Center, Omaha, NE
- 10:05 AM–10:25 AM **Break**
- 10:25 AM–11:05 AM **Is the Two-Year Rodent Bioassay Needed to Address Carcinogenic Risk for Human Pharmaceuticals?**
Frank D. Sistare, Merck & Co Inc., West Point, PA
- 11:05 AM–11:45 AM **A Weight of Evidence Approach to Cancer Assessment**
Alan R. Boobis, Imperial College, London, UK
- 11:45 AM–12:45 PM **Roundtable Discussion: How Can the Rodent Bioassay Evolve to Meets the Need of Predictive Toxicology?**

Moderator: A. Wallace Hayes, University of South Florida and Michigan State University, Temple Terrace, FL
Panelists: **Todd Bourcier**, US FDA, Silver Spring, MD; and **Janet Zang**, US FDA, College Park, MD
All Speakers

US FDA Welcome and Overview

Admiral Denise Hinton, Chief Scientist, US FDA, College Park, MD

Suzanne Fitzpatrick: I can open it up. Thank you very much for all of you here today on our meeting, Redesigning the Rodent Bioassay for the 21st Century. I am very happy to introduce Denise Hinton, FDA chief scientist, to give some opening remarks. Thank you.

Admiral Denise Hinton: Thank you all. Can you hear me?

Fitzpatrick: Yes.

Hinton: Great. I want to say good morning everyone. I am delighted that most are able to participate in today's meeting despite challenges with travel and the like you to weather. We welcome challenges at FDA particularly those brought about by those in science and technology. Science is all about developing new tools and assays and working with stakeholders. Advances in biology, stem cells, engineered tissue and mathematical modeling are creating unique opportunities to improve FDA's predictive ability, thereby enhancing our ability to predict risks and efficacy. These risks are expected to bring FDA products to market faster or with increased technological risk from reaching the market. And to evaluate new methodologies, predictive capabilities and replace, reduce or refine animal testing, FDA senior toxicologists from across the agency including those from the National Center for Toxicological Research developed a comprehensive strategy which is FDA's Predictive Toxicology Roadmap.

We then turn to our stakeholders in academia industry in our federal partners in September of last year in our first public meeting on the roadmap to solicit input on how we could work with them to spur the development and evaluation of new technologies and incorporate this into our regulatory review. Factoring collaborations across sectors is a key component of our six part roadmap. FDA considers its partnership with the Society of Toxicology pivotal with the community. We need to redesign the rodent chronic bioassay to meet the needs of the 21st century risk assessment. That requires an open, transparent, science-based way of updating our regulatory tools. That involves stakeholders as we go forward. I look forward to hearing the results of today's meeting and hope that this is the beginning of further discussion with all of you as we work together in protecting and promoting public health while creating a safer and healthier world by advancing the science and increasing the impact of toxicology. Many thanks to our co-chairs Suzie Fitzpatrick of the FDA Center for Food Safety and Applied Nutrition and to Warren Casey of the National Toxicology Program at the National Institute of Environmental Health Sciences for hosting the scientific conference today. Thank you to all of our speakers for your commitment. With that, I will turn it back over to you and congratulations. I'm sure it will be a successful meeting.

Welcome from SOT and Introductions

Suzanne Fitzpatrick, US FDA, College Park, MD

Fitzpatrick: Thank you so much. As she said, as toxicology changes, we as regulators at FDA want to look at our regulatory tools and see how we can advance them to meet the challenges that we all face in the 21st century. I will change hats right now and get some opening remarks from a SOT if we can have the next slide. This colloquium series which has been a big success has been a partnership between the FDA Center for Food Safety and Applied Nutrition and Society of Toxicology and it is really just stimulated dialogue amongst tox experts on how we can make toxicological science relevant not only for food and food ingredients but for all products that FDA oversees. It brings information to FDA employees and the public. It allows a form for us to talk about science, not giving regulatory advice or regulatory issues to discuss the latest tox science with our stakeholders so they will all come to a consensus on how we should move forward.

I think that is it. Welcome. The mission of SOT is to create a healthier and safer world by advancing the science and increasing impact of toxicology. And among the strategic priorities are strengthen the relevance and impact of toxicology, develop and support toxicologists to capitalize on future opportunities and expand outreach like we are doing here globally to get all of our stakeholders important dialogues of how to advance toxicology in the 21st century. Next slide.

This is the fifth year. We had a wide-ranging number of topics. We had a global audience from over 300 webcast registrants for more than 17 countries. We find it is a good resource for 24/7 learning. All of the slides from this symposium and all of the other symposium that we have are free for you if you access our website, www.toxicology.org. Next slide.

Here's a list of the organizing committee, Bryan Delaney from the Council is our chair along with Allen Rudman from CFSAN. As you can see, Jason, myself, Jieun, Steve, Jieun, Jeff and Patricia. We all discuss and have impact on this and other programs. Next slide.

I guess that is it. I think there was a slide about the next webcast. Maybe not. So, with that, I'm going to turn it over to Wally Hayes to introduce our speaker. Are you there?

A. Wallace Hayes: I am here, Suzie. Good morning. We are just delighted to have this opportunity to relook at the rodent bioassay under the topic Redesigning the Rodent Bioassay for the 21st Century. We are very thankful for the Food and Drug Administration and the Society of Toxicology for putting together this session, and we have four outstanding speakers that will share their thoughts with us this morning and I'm going to introduce all four of them at the same time, and they will make their presentations in the following sequence. We will start off with Doug Wolf who at Syngenta Crop Protection. He will talk about the chronic cancer bioassay as frequently conducted for pesticides when it is not always needed to protect humans. This will be followed by Sam Cohen at the University of Nebraska Medical Center who will talk about threshold based risk assessment is the same for cancer and noncancer endpoints, for non-DNA reactive carcinogens, and then we will have a break, and I will come back and introduce our last two speakers at that time. So, I will turn the program over to Dr. Wolf. Doug, are you there?

The Chronic Cancer Bioassay Is Frequently Conducted for Pesticides When It Is Not Always Needed to Protect Human Health

Doug Wolf, Syngenta Crop Protection Inc., Research Triangle Park, NC

Doug Wolf: Yes. I am here. Can y'all hear me okay?

Hayes: I think you need to move closer to the microphone.

Wolf: Okay. Can you hear me? I am not on mute.

Technical Staff: We can hear you. You are just a little far from the microphone.

Wolf: I shouldn't be. I am about as close as I can get.

Technical Staff: That right there works. Thank you.

Wolf: Are you going to give me control of the slides?

Technical Staff: You already have control.

Wolf: Oh okay. I want to thank the organizers for the opportunity to come talk to everyone. This will be interesting with everybody on the webinar. Always a challenge. It is a challenging topic, so I think we are all up to it.

There. The obligatory conflict of interest, I will move on to our first slide giving an overview of what I intend to talk about today. To set up the idea that using our accumulated knowledge from exposure so the use drives the exposure, the physical components of the molecule drives the interaction with the environment as well as the biological systems. So that again impacts exposure and the idea is to focus on what questions need to be answered to protect public health from cancer risk. We will address the chemical series of papers that a group of us recently published looking at a unified theory of carcinogenicity bringing together the past 40 years of research and modeling of idea around cancer including where the hallmarks of cancer fit in, the idea that we can predict the potential for human carcinogenesis without having to kill a bunch of animals to do it.

Put it in context of a transparent systematic approach that is focused on what is the problem you are trying to solve and what is the exposure to inform that problem. Give an example of how the US EPA is implementing their waiver program over the past number of years, very successful program. Again, it is a precautionary approach that allows you to make public health protective decisions without necessarily doing excessive testing in the sense that we don't need to kill a lot of animals in order to inform the public health decision [indiscernible]. I will give you an example on where we are moving ahead with developing and guidance for the waivers for cancer outcomes. And a potential path forward.

I am getting used to doing this remotely. Every web tool works a little bit differently. So, when you think about the exposure, the idea that humans are exposed to contaminants in the environment, they have to actually get to the target population, the individual, and the target site inside that individual to interact with that biological system and alter it sufficiently over a sufficient period of time to result in an adverse outcome including cancer. So, when you think about the importance of exposure, bringing in the idea of exposure into can the [indiscernible] chemical get to the individual, how it gets inside that individual, what the blood levels are, what the tissue concentrations are, doesn't impact how it interacts with the internal biological site,

receptor, or some other macromolecule? Then how does the cell respond to that? Does it adapt? There is a lot of background noise. If people will put you are phones on mute, I would appreciate it. Does the cell adapt to that response over time? And maintain its function? In this new environment with this chemical exposure around it. How does it change? What toxicity pathways would it perturb and then what happens to get it transformed into a cell that can survive, proliferate, [indiscernible]. What are those components? We know that hereditary factors are very important so there are some tumors and cancers that result, that can influence, are the result of inherited genes. Certainly, cellular replication is very important so cells, replacement cells, either due to damage or just stem cells replacing the normal constituent of senescence and loss of cells so the [indiscernible] replication is very high in certain cells and very low in other tissues, almost nonexistent.

Then there is external environmental factors. There are chemicals that interact directly with the DNA so they can be genotoxic and bimetal factors and then there are [indiscernible] inducing cytotoxicity [indiscernible] or buying the receptor and stimulating cells. At the cellular level, we know that cells proliferate. They can change the DNA. If the cell has too much damage to it, the cells can die and that there is a repair factor. So, cells can repair themselves as well. So, what we have done in this model is to bring together the models that have gone before [indiscernible] that others have done over the years, there is a lot of background noise. Is somebody cooking breakfast? It would be nice if that would go away.

Technical Staff: If you are on the phone, can you please mute your phone? Please mute your phone. We have sent messages to people that we have seen better unmuted. Can you please mute your phone?

Speaker: Particularly the person that is shuffling papers in the background, it is getting very annoying.

Wolf: We have to be adaptive like the cells, so we will adapt to the situation. What we have done any series of papers is to bring together all the work that has gone before and expanded the model. When you think about carcinogenesis, is fundamentally due to mistakes in the DNA that accumulate and what is necessary is more than a single mistake, so a single genome is not going to result in cancer even when you think about hereditary cancers. It takes more than one event to result in the final adverse outcome. The mistakes have to accumulate. It adapts to a sustained stress environment and survives and proliferates so this is a high bar or a cell. The cell population at risk are those cells that actually divide so many of them are stem cells that can repair and divide. There is every time a cell divides, there is some opportunity to the for the DNA to not be accurately replicated. So, you can have mutations that occur. So, think about carcinogenesis as a process. Is really a random event. So, it is not directed activity that will always lead to an adverse outcome. It is a random process. So that randomness is part of the model you have to think about.

As we tried to put together the different components, and in this particular example, you have a collection of six mutations that need to occur for the ultimate initiated cell here at the end to survive and proliferate. So, each time that this event can happen, you look at the original ancestors cell as it proliferates. There's some risk of a mutation to occur. Three is a typical number. There is repair going on. So, there is a good chance that all of the mutations would be repaired, but through a stochastic process, there may be an acute relation of a cell mutation in that gene. That doesn't necessarily mean that gene has any impact so when you think about the 30+ thousand genes that exist, that may not be any gene related to any kind of control mechanism that is related to proliferation or inhibition of cell death or any of these things that

have to occur in cancer. So again, this is a random process. Or the amount of change in the genome could be so significant that the cell dies.

So, this repetitive process has to occur and each time in a sustained stress environment where the cells in the organ are exposed to environmental encounters that might be immunogenic but also proliferation, those have to be present. They have to be present consistently and therefore a long period of time. You also have of course hereditary factors and the constituent of replication also helping to drive this process. Ultimately over time, if again, you are continually exposed to these environmental factors that are driving either gene toxicity or replication, you can possibly end up with a cell with sufficient numbers of changes in their genome that allows it to survive and proliferate.

So then, with continual exposure to the chemical, it is maintaining this internal environment that is continually stimulating these cells, you can get proliferation into a [indiscernible] focus or cells that contain those series of mutations that encourage that cell to survive and proliferate and with continued exposure or continued replication either constitutively over a long period of time or an environmental contaminant that is of sufficient exposure for a sufficient duration of time that is stimulating these cells to continue to proliferate and move toward malignancy. So, this is a process that requires a number of different factors and for chemicals, we can understand a lot of these components that process. So, the important components of that process are laid out here in this diagram which are identifiable and quantifiable and therefore, allow you to do better predictive evaluation of the potential for human carcinogens. So, remember, we are not necessarily worried about cancer in wildlife. That is a different part of the risk assessment. We are focusing here in this discussion on human cancer and identifying the potential for human cancer. So, we understand a lot about what causes cancer in humans.

You can then therefore ask in a very rational way, does the chemical concern, are your chemicals of concern causing any of those features that can result in the potential for humans to develop a tumor? So, is it mutagenic? Yes. Then exposure. What is the exposure? Is it about the threshold of toxicological concern? If it is, then what is the metabolic pathways of the parent metabolite that has mutagenic potential? Does that metabolism likely to occur in humans, at what level? And then you can perform a risk assessment and set permissible exposure levels. If it is below the threshold of toxicological concern, then there is no toxicological risk and you no longer need to worry about the cancer.

For a 90 mutagenic chemical, there is a series of questions you can ask that are available and it is called [indiscernible] classifications where you can catalog based on the structure and many other features of the molecule and what is known about the potential toxicities of different classes of chemistry and two different Cramer classes. I won't go into any more detail on that. You can view that your leisure. If there is a Cramer classification, then you can look and see it again what the exposure is, the threshold of toxicological concern. If not, then you know there is no more evaluation needed. If it does, then what is proliferative activity in the various target tissues of concern? What do we know about the target tissues and in short and intermediate term studies, what is the cell proliferation and the dose-response? Does it have endocrine activity? What are the thresholds? Is an immunosuppressive? We have these effects, but these effects occur, and we know that the motor action is indeed relevant for humans. Sometimes it isn't. But if it is, then using properly conducted studies, looking at dose-response and no effect levels, you can quantify and calculate the reference concentration using different tools, either using a no adverse effect level and uncertainty factors or benchmark dose modeling. Then using those outcomes and points of departure to form a risk assessment to set permissible exposure levels. None of this requires a two-year bioassay. All

this is quantitative and well understood and helps protect the [indiscernible] risk harm a determination of a chemical.

As we think more holistically about how one approaches this, the Health Environmental Sciences Institute put together a project which we call Risk Assessment in the 21st Century or Risk 21 for short. There is a website which focuses on first DD their problem formulation. What is the risk management decision you are trying to inform with the data you are trying to collect in order to protect public health? So that is really critical. Then the second is what is the exposure? As I described to you just a moment ago, exposure is key. If you understand the exposure, the risk of exposure, how I can get to the target population, you can do a much better job of predicting whether or not there is even a hazard to be concerned about. If there is reasonable exposure, then you look at the toxicity. This flips our traditional way of working on its head a little bit. You think of others [indiscernible] both external and internal concentration, and then worry about hazard. Typically, as toxicologists, we do a lot of studies and find a lot of hazards that we don't really have any way to relate that to what the real problem is. So, this is really critical for the problem formulation.

Once you understand that, there is a two on the website that helps you to pull this information together, what you know, and then asks the question what do you need to know? Explore a bit more. Would an additional study make any difference? Depending upon what you find in your evaluation of the exposure evaluation as well as the hazard evaluation, you may find that additional study would have no impact. Depending upon what you learn from the evaluation, it may be that understanding exposure better will be the best driver of solving your risk management problem or maybe understanding the hazard and its human relevance. So, the tool here allows you to have that discussion within the context of your risk management decision without immediately doing a bunch of studies that may not provide any specific value.

All papers are available on the website, freely downloadable and I encourage you to take a look at those, including how you would quantify this in a community risk assessment. So, the key part of all of this is really the problem formulation. Having the right discussion with the right people. It is not just an individual doing it together. You really need to think about what are the decisions you are trying to inform, define the problem, examine it, come up with the appropriate problem statement and then explore. What do you know? What questions do you need to answer? What are the hypotheses you need to test? Come up with a conceptual model. And then only then, can you map and approach to solve the problem. At each point in this, you make sure that you are actually solving the problem as defined.

So, these are relatively new publications but quite frankly, this is the approach the US Environmental Protection Agency has taken for many, many years in evaluating crop protection. There are as we all know legislation and required testing that exists. That is in order to ensure that any product use in agriculture is safe for used, fit for purpose, and that the way it is being used is health protected from both the operator, the person using it in the agriculture field as well as the ultimate consumer. However, there is flexibility built into the system. That is such that the US EPA test site program can require additional studies, more detailed studies, that are either listed in the guidelines or alternative studies that may be more mode of action or other types of studies to really understand what might be driving an adverse outcome. Also, they are able to accept new approach methodologies and most importantly, if they already know the answer through the data that they have and the knowledge they have, then you don't need to kill a bunch of animals to answer your question.

So, the guiding principle is what do you know? What do you need to know in order to protect public health? This is the guiding principle we codified within the Risk 21 framework, starting with a risk management problem. What is it you are trying to solve? What assessments do need to support? So again, starting with the problem formulation. Then you only require data that are adequately informed regulatory decisions.

So, if you have a data lead, not a data gap, that is a science discussion, a data need, a regulatory decision, even identify what study most appropriately will support that. Sometimes you will need to, you may have a situation where a cancer bioassay might be warranted but it comes out of the context of the problem formulations [indiscernible] exposure. I would argue that that would be a [indiscernible] in the future. The value of this is that it avoids unnecessary use of time and resources, not just the time to do the studies comp at the time to evaluate them. So, when you think about the difference between the time that is invested by a regulatory science at a regulatory agency to look at the huge amount of data, hundreds of pages of information in the cancer bioassay and that provides them no value in protecting public health, this is a great savings to taxpayers without diminishing public health protection. So, it is a valuable approach to again, support the evaluation of products that are fit for purpose.

While we are just specifically focusing on the cancer bioassay for crop protection chemicals, there are large numbers. These are the series, nine series of tests. Each series has multiple test guidelines. We are only going to talk about the age 70 series. When we talk about the cancer waiver in a few minutes and what goes into it, all of the data that we collect for these chemicals goes into that evaluation. So, the question then becomes do we need this one additional study? Do we have enough information we look across the [indiscernible]? If you look at the H 70 series, we are trying to understand what is the acute high dose toxicity? This is always very important because there are accidental exposures to an irritant. Is a corrosive? All of those things that can happen with exposure. There is also a large number of short term and intermediate studies. Some are required based on how it is used and so the use is really important. Is it just going to be a herbicide that is used one week a year and there is no food exposure? That this may be a different set of [indiscernible]. Will it be antifungal on apples that people are exposed to? That is a different kind of set of data. Understanding again the exposure is absolutely critical. How it is used, what is the indications of herbicide and insecticide and fungicide? That drives the data collection.

So, you have all these intermediate shorter-term studies and ultimately, the one we are talking about today is this carcinogenic one. As you can see, it is already part of the conditional requirements for non-food use. [Indiscernible] because of what we know about cancer and carcinogenesis, it isn't required for that. And of course, as I mentioned earlier, the first step in the process, is there any evidence that the chemical of concern can directly interact? So of course, there are a number of assays to evaluate that potential.

Waiving studies is not new or unique to the EPA. There are global guidance on acute toxic testing so those of you who aren't familiar with pesticides, there is an active ingredient that is put into a mixture of other types of materials called co-formulates that allow it to be used in the environment so you may have something to kill those weeds but you have to be able to sprayed on the weed. It has to stick to the lead and there are other materials in that final product so those change quite often. There may be hundreds of those. There are hundreds of herbicides used differently and so there are acute toxic testing but a lot of them have very similar formulations. We have historically waived those acute toxic studies. A lot of animals are killed in order to do the acute toxic testing.

One of the things that has been implemented globally is if we already know about this particular formulation and the changes and it aren't that significant and you can make that comparison, then we don't need this particular study. So that has been around for some time in your guidance globally. What we are talking here with the US EPA is the forward-thinking data move and thinking about repeat dose studies and not just acute talks but also some of the longer-term studies including inhalation studies, pheromones, immunotoxicity, neurotoxicity studies where we can already know the answer, we don't need to answer the question.

So, how successful, the waiting repeat dose studies was implemented as a formal program in 2011. As registrants, we can submit waivers. It is a high bar as it should be. Can we answer the question that that particular study was designed to answer using all the knowledge we have gained from our development process as well as all the testing that we have done, not just for the active ingredient we are talking about but others in that class or others in that use pattern? It is a scientific activity? Do we have sufficient understanding and knowledge to inform the risk decisions? What is the problem formulation? What is the risk management decision you are trying to make and to we already know the answer? You pull all the information together and bring in the knowledge of whatever else is necessary to inform that decision. It avoids the [indiscernible] data. The agency is not looking at a bunch of stuff that has no value to it. They are not wasting their time and they are not wasting taxpayer dollars on looking at data that doesn't inform the risk decision.

It is a very scientific approach because you need to go back and look at everything. When you think about the computational toxicology program and what the intent of those kinds of activities are, it is always about what you know and what you need to know? When you think about all of the computer data, the high-content data, the large amounts of data we are trying to pull in, even what Facebook and Amazon does and you can go on, they have pulled all of this data together. You type in I want this book and people say what people that buy this book have also bought these books, you go I am interested in those as well, they pull all this data together. We are trying to do the same thing with what do we know about all of the chemicals?

In the waiver program, you pull all of that together to understand do we need this particular study? Frequently the answer is well no, you don't. You can already answer the question to protect public health and the environment. The other important aspect of this is they are much more focused activities to write a waiver. It is a much more focused activity to evaluate the waiver. So, it takes less time. That is great because a farmer in the pesticide program used to say decision delayed is protection tonight. It is really important. We want to get those decisions as quickly as possible. Also, to remember is for the pesticide program, we are trying to provide innovative products to the farmer, to the public, to improve agriculture and protect us from malaria mosquitoes and that kind of thing. Decisions delayed is innovation denied. So, you want to get to those decisions as quick as possible. So, being able to waive the studies is really important. It relieves unnecessary testing.

So, we presented this as a poster back in 2017 in collaboration with EPA and the Registry community and this was a part of the poster. If you look at the success of the EPA's waiver program, again, you need this study? Can we adequately protect public health without killing these particular animals? And frequently, the answer is yes. We know enough to do that. If you look at just this collection of waivers, and this number has gone up dramatically since 2017, what we have shown here, and this is just the guideline studies. You have to remember that there are also preliminary studies that are done in order for you to do the guideline studies so this is a very conservative low number estimate of the savings here. A tremendous number of

animal savings and tremendous amount of dollar savings for this. This doesn't tell you the savings of the agency in time and resources not to have to review studies [indiscernible] to the final health decision.

So, we start out with the problem formulation. We went through with a collection of people. We come up with the problems that are conducted by assays. We came up with a key knowledge needs as the path forward, the conceptual model and the path forward on what we need to do. We came up with the criteria, food use pesticides.

These were the criteria that we came up with. Again, exposure. How is it used? How is it acting in the environment? What other metabolites? How does it get into the target? How is a change in metabolism and then the effects studies? What does it do *in vitro* and *in vivo*? Does it interact with DNA, special studies including cell proliferation? Does it suppress the immunosuppression, going back to that framework presented earlier and what you know about the other chemicals in this class? It is about exposure. There is a margin of exposure adequate to do the risk assessment.

So, we went ahead and did an example. I created a draft waiver on a product recently submitted to the agency. It was approved for use, so we had all the information. We evaluated based on the criteria. This is the table of contents. You can see that we looked at exposure, internal concentration, all of the testing that was done including some specific mode of action studies, all the different features that could occur that we know are related to cancer in humans, looking at the other molecules that have been approved for use in that same class and of course, exposures are important. I way to look at this number here. This is a waiver documents. This one was 23 pages with references. Some are little bit longer, maybe 30-40 pages, others may be shorter depending on what kind of waiver you are writing. That pales in comparison to the number of pages in a typical cancer bioassay. If you are a regulatory scientist and you have the opportunity to make a decision on public health protection, and you only need to read the summary of a lot of work and to know that that work is done and what the conclusions of that work are and you have access to all of that work, it is a much more straightforward process and much less time-consuming than reanalyzing the individual data from a cancer bioassay which provided you no additional opportunity to protect public health.

Again, this approach provides the information necessary to protect public health without wasting time and resources. We learned a lot from the example that the EPA has evaluated and came up with a preliminary evaluation. I want to make sure that we have sufficient information, the exposure potential. That is really critical. Again, but the extra exposure, how it gets to and then the kinetics, how it distributes. Doesn't get to the target tissue? What other concentrations in the blood and target tissue, what are the relevant toxicities? Are we getting the consistent data with what we know about internal concentration elimination? Of course, concerns about genotoxicity are very valid. Again, this is a high bar as it should be but if you do adequate testing early in the development process, focus on what is necessary to be able to protect the potential for concerns around human tumor developments, then you can have the sufficient information to waive those things.

So, in summary, we have the access to a range of *in vitro* and short-term *in vivo* assays that can be used to evaluate the carcinogenic potential. We understand the carcinogenesis process and the framework of how you would address the different things that would cause human cancer. I know in Sam's presentation, he goes into much more detail of what does that look like. You should be able to identify affects that mainly directly or indirectly to any changes. That is critical. Exposure. Risk managers, the only decision they can do is limit exposure so using

this Risk 21 approach really gives you a better handle on what is the exposure necessary to be concerned about various adverse outcomes including cancer. In the case of most of these products, because the products that get on the market and the crop protection industry have already been screened out for the immunogenicity component. We are talking about things that can cause chronic toxicity. If you can control the chronic toxicity and prevent that by understanding the no effect levels or the driver effects, then tumor development is just another indicator of chronic toxicity.

These are happening, doses that are causing other toxicities. You can prevent that just by identifying the no effect levels or the drivers for the chronic toxicity. We know a lot about mode of action that can turn in not just the mode of action of the [indiscernible] but we also need to understand the pesticide mode of action and relationship between how that pesticide works and the target population, whether it be plants or fungus and the relationship between that in a target to what would happen in animals. So, because of all this unified theory, we do not need a separate category for carcinogenicity. In these cases, understanding how chemicals can perturb the system sufficiently to result in tumor development and just having a risk assessment to prevent adverse consequences, it is really all that is necessary. Again, tumors are just outcome of chronic toxicity. Assessment based in this approach will still safeguard public health. When you think more broadly of a large number of chemicals that haven't gone through to you or quantified assay in the toxic space and that the innovation that we are trying to bring to the community, cancer bioassay really do not [indiscernible]. It is costly and unnecessary.

So, my conclusions here are that the long-term bioassay is really not necessary to evaluate potential for carcinogenicity in humans. Has only classification just does not provide any public protection and that we understand a lot about human carcinogenesis today. Utilizing mode of action analysis in a more direct and rational basis for human cancer risk assessment, the framework I presented earlier, based on these modes of action and can be performed rather than simple hazard identification. So, understanding the exposure mode of action. It avoids this waste of time and killing animals unnecessarily. We end up being equally health protected to prevent the adverse outcome of from chronic exposure. In the case of almost all of these cases is that tumor development is just another example of chronic exposure, persistent chronic exposure. So, with that, the other slides here are references. I will stop here. There might be a little time for clarifying questions. I know we have a panel discussion leader for any in-depth discussion.

Hayes: Thank you, Doug, for a great start to our session and your timing is just perfect. We have got time for one question. In fact, we do have one question that has come up. That question is, what is your view of a change in epigenetics as a mechanism to carcinogenesis for non-genotoxic agents. Is it or should it be assessed?

Wolf: I think in the context of the framework we have identified is you have to say well what is that epigenetic effect doing? That is a mechanistic question, but the mode of action question is still embedded in the framework. So, is that epigenetic change driving increased [indiscernible]? You can quantify that and come up with a no effect level. Just because there is in epigenetic change doesn't equate that that changes the paradigm. It could be happening, and it might be something you're interested in more from a drug evaluation, a drug target perspective but for our predictive cancer risk point of view, that would still be folded into how a cell responds and stimulates persistent uncontrolled proliferation. Then, what is the no effect level for that? It may be interesting mechanistically, but it is still embedded in a framework that I have presented earlier.

Hayes: Thank you for that answer, Doug. Don't go away. Remember, we have our panel discussion.

Wolf: I'm not going anywhere.

Threshold-Based Risk Assessment Is the Same for Cancer and Non-cancer Endpoints for Non-DNA Reactive Carcinogens

Samuel Monroe Cohen, University of Nebraska Medical Center, Omaha, NE

Hayes: We are right on time. So, Dr. Sam Cohen, you are up to tell about the Threshold-Based Risk Assessment Is the Same for Cancer and Non-cancer Endpoints for Non-DNA Reactive Carcinogens. Welcome, Sam.

Samuel Monroe Cohen: Thank you. Can you hear me?

Hayes: Yes, sir.

Cohen: Thank you and thank you to the organizers for this very interesting topic. If I could have the next slide. This is the standard conflict of interest statement. Mostly regarding the FDA, I want to mention that I am a member of the FEMA expert panel which does the safety evaluations for flavoring ingredients. Here is the current funding that I have as well. Next slide.

My talk is going to start with the basic principles of carcinogenesis and then focusing on how we can extrapolate from animal models to humans based on mode of action and human relevance analysis. Then looking at the methods that are used for screening for cancer risks particularly on the lack of need for doing a two-year bioassay. Then focusing on it genotoxic carcinogenesis in particular. I'm not going to go into any depth over DNA reactive carcinogens and then utilizing the inorganic arsenic in drinking water as an example of how to apply a non-genotoxic risk assessment and then some conclusions.

I want to emphasize that this is not a new model. I think part of the difficulty we have had over the years in screening for various toxicities is that we have focused on the technology without focusing on the biology. It is very easy these days to become infatuated with these high-tech that we have available but that is only useful if we apply it in a carefully understood biological basis to just do the technology is going to lead us astray. So, this slide which Doug also used gives the basic principles of carcinogenesis. This is not a new slide. This is actually almost 40 years old and was based on a slide that we produced back in 1981 when we were trying to develop a model of carcinogenesis based on animal data. We are developing one based on epidemiology which was very similar. Ultimately, it goes back to basic principles that were outlined by AI [indiscernible] in his seminal 1971 [indiscernible] paper which essentially outlined to these basic principles. These are not new. The problem is we have been ignoring these basic principles and just looking for a magic bullet to screen for various toxicities, in particular cancer.

Based on these basic principles, there is fundamentally only two ways that any agent come a chemical or otherwise, can increase the risk of cancer. One is you can damage DNA directly such as with [indiscernible] hydrocarbons, etc., or you can increase the number of cell divisions which leads to an increase in the number of spontaneous mistakes that can occur

during a normal DNA replication. Again, these all have to happen in a single cell. It is a totally realistic problem. We have to stop thinking if we are exposed to chemical A that we are getting cancer B. If we are exposed to a certain chemical A, there is a certain probability that you will develop cancer B. This should be obvious to everyone. For example, with cigarette smoking. Even a three pack a day cigarette smoker only has a 10% risk of developing lung cancer during their lifetime. That is not very high when you put into perspective overall and yet, people still understand that cigarette smoking is carcinogenic. Next slide, please.

Is so fundamental for modes of action of human carcinogens can be narrowed down to these four fundamental principles. One is DNA reactive, I mentioned a few of these. It is immunosuppressive. We have a number of things that do this. This can be either a drug treatment. It can be, is somebody eating in the background here? Can you please put your microphone on mute? It can be an inherited immunosuppressant, drug-induced, cancer therapy, autoimmune therapy, whatever. You can have AIDS. The consequences are still the same. You have an increased risk of cancer but not just any cancer. These are the virally related cancers such as EBV-related lymphomas, HPV-related squamous cell carcinomas, etc. Estrogen is the only mitogenic agent that we know of that just based on straight mitogenesis without cytotoxicity increases the risk of certain cancers and then we have a cytotoxicity and regeneration which I will focus on shortly but all of these essentially are either DNA reactive which is the first one or the others all lead to increased cell proliferation on a chronic basis.

So, in animal studies, this is very common. Here's a list of just some of the modes of action that have been identified regarding various modes of action, increased cell proliferation, either direct mitogenicity or cytotoxicity regeneration. All of these are going to have a threshold-based to their mode of action and to be honest, on the mitogenic side of things, the only one that is relevant here to humans is the estrogen-based ones. The ones on the right are others. Fundamentally for the animal bioassays, we have come a long way in our understanding of what can cause cancer and the dose response is necessary and most importantly, even relevant to humans. Next slide, please.

So anytime we do an experiment in animals and fundamentally in any model even *in vitro* or *in silico* is that we are making certain assumptions with regard to cancer. To be honest with you, any toxicity. One is that the effects that the high dose will occur at low dose. Industrial chemicals and food chemicals, the difference between extra mental dose and the human exposure levels are quite vast. In contrast, pharmaceuticals many times, the exposures and the animals are not that much different from the exposures and humans. The second is that the effect that we see in the rodent will extrapolate to the human. This is species extrapolation. For these two modes of action for DNA reactive carcinogens, these are reasonable assumptions, not perfect, but they are reasonable. For non-DNA reactive carcinogens, one or both of these assumptions may be inaccurate and totally incorrect.

So, how do we extrapolate from the animal models? This is the IPCS Human Relevance Framework which is built upon that which was developed [indiscernible] sponsored by Health Canada and US EPA. You start by saying is the weight of evidence sufficient to establish the mode of action in the animals to begin with? Many times, we actually can identify the mode of action reasonably readily using genomics, we can do this fairly quickly and eliminate a number of others. The important part is that you not only evaluate the mode of action you think is operational, but you evaluate all possible modes of action and try to indicate why other modes of action are not pertinent. Once you have a mode of action, then you can look at the qualitative and quantitative aspects of each of the key events in these to see if they extrapolate to the human situation. Then you have an overall statement of confidence analysis and the

implications for the risk assessment. This is outlined a number of publications and this continues to evolve over time. It is a very I think disciplined approach to looking at the human relevance and being able to evaluate the animal data. Next slide, please.

So, the two-year rodent bioassays, we are all familiar with the difficulties of its. It costs a lot of time, a lot of money and a lot of animals. You also cannot do very many doses. Generally, two, three, or at most for any long-term bioassay. The two-year bioassay by itself doesn't tell you anything about mode of action. Although, the findings can be helpful in narrowing down the possibilities. Most importantly, the two-year rodent bioassay doesn't really tell you anything about human relevance. You have to do other studies to do this. And fundamentally because of all of these, people now and for a very long time have questioned about the applicability of the two-year rodent bioassay screening for carcinogenesis, this is originally developed nearly 60 years ago on the assumption that because most human carcinogens are positive in the rodent bioassay, things that are positive in their own bioassay must be human carcinogens. That leap of logic is totally inaccurate because we now know with considerable experience over the last 60 years that there are many things that happen to rodents that are totally irrelevant to humans. The fact is I'm going to point out with regard to cancer, the rodent bioassay is virtually useless. Next slide.

So instead, we can do much more detailed analyses utilizing shorter-term assays and utilizing what we already know from the vast literature that as Doug pointed out that is available on a number of chemicals overall. In a detailed 4- and 13-week bioassay, there are a number of signals that indicate the possibility of a cancer risk in the animal model. One is an increase in organ weights, frequently see this with the liver for example. Histologic evidence of toxicity and/or proliferation. So, hyperplasia for example, blood and urine chemistries, DNA labeling indices, and then there are some specialized studies that can also be performed although I don't think these need to be routine. I foresee all makes replacing a number of these things as a screen because you can identify not just the one mode of action that you are looking at, but you can exclude a number of others as well. These are all critical. Next slide, please.

Here's a bunch of tumors that are identified in rodents that are really totally irrelevant to human risk assessment. I just listed some of these. There are a number of others, there are some organs that just have no human counterparts to why bother looking at them? There are some that have no human analog. Endocrine tumors generally yes, it is important to find endocrine toxicity, but they are not predictive of endocrine carcinogenicity except for estrogen. That is really the only one. All the others of these, there is no predictive value with regard to human carcinogenesis. Then also, reproductive endocrine tumors also in the rodents are not predictive for humans. For example, the granulosa cell tumors that are common in the ovary, Leydig cell tumors in the testis, endometrial tumors, prostate tumors, these tumors are totally different in the rodents than they are in humans and none of these have ever predicted a human carcinogen other than estrogen with regard to the endometrium.

So, screening for carcinogenesis is relatively simple. We can work through this diagram. We have a chemical, the first thing we do is evaluate it for DNA reactivity. If it is positive, that is going to raise concerns and we have to focus on whether it is DNA reactive in humans or not and is going to be based on a metabolism. If not, we can then evaluate it for immunosuppression. These are relatively easily done in short-term assays. You don't need a long-term and I don't mean that this is just immunosuppressive. These are clinically relevant immunosuppressant estrogenic properties. If these are positive, again, US media something that these are relevant to humans.

But if it is not, you have to evaluate other possible sites of increased cell proliferation. Again, not worrying about a number of tissues because it is not going to be relevant to humans except for the toxicity. Certainly not for cancer. If yes, you can do mechanistic evaluations and you can identify the mode of action and detailed dose-response and you can come up with whether this is relevant to humans or not. Both with regard to dose and even more importantly mode of action. If it is not relevant to humans by mode of action or dose that we don't have to worry about cancer with these. I will go into some details of how this can be applied in the basis of the example of inorganic arsenic. Next slide, please.

In this simple screen, if there is a positive, one can do these more detailed studies to really narrow down what the dose-response is, the mode of action and even assess what the human relevance of the findings in the rodent are in the first place. Again, focusing on human cancer. So fundamentally, we have to stop doing the two-year rodent bioassay. But it is important thinking about ending the two-year rodent bioassay is that we are going to have to make some effort into how we label chemicals after this. There is going to be a lot of false positives based on the rodent bioassay. For example, increased liver weight. That happens a lot, but it has nothing to do with carcinogenesis. We are going to have to really begin working on how we are going to label chemicals that are positive in this screen and come up with a better conveyance of the information to the public that says yes, this does this in rodents but it is not relevant to humans even with respect to the mode of action or if it is not relevant with regard to human exposure levels. This is particularly true exposures with some of the things like pesticides and a number of other very low-level chemicals such as the flavoring ingredients that seem expert panel frequently evaluates.

For non-genotoxic carcinogenesis, going back to basic principles. You have chemical exposure, you get a noncancer toxicity which is a required step in the process to developing cancer. This noncancer toxicity leads to an increase in cell proliferation. It ultimately leads to tumors. These are the principles that we start with non-genotoxic carcinogens.

The key is that all of these go through a requisite precursor toxicity lesion which is produced even by direct mutagenicity or cytotoxicity and regeneration. These pre-cursor toxicities can be detected in short-term assays. You do not need a two-year bioassay to detect this. You have to do more and these short-term assays than what we currently do so for example labeling index may be necessary to pick up increased in a proliferation that is not obvious on a [indiscernible]. Structure activity relationships are very useful. A number of other things too. The other is that the dose-response for these genotoxic carcinogens is going to involve a threshold. What is the relationship between the dose that is required for producing this precursor lesion and the exposure in humans? This was first pointed out in some detail for chloroform and chlorinated drinking water to show that the cytotoxicity that is produced by chloroform at high levels is totally irrelevant to the low levels of chloroform present in drinking water. So fundamentally, it comes down to if we protect against these precursor noncancer toxicities, we will protect against cancer. So, the risk assessment for the cancer is extensively based on the noncancer toxicity. So, we don't have to do a totally different cancer risk assessment. Again, I am focusing here on non-DNA reactive carcinogens. For DNA reactive carcinogens, that is another story. Then I want to focus that it really is DNA reactive, not all these indirect genotoxic mechanisms frequently which don't translate to the *in vivo* situation.

Let me now use inorganic arsenic in the drinking water as an example of a non-DNA reactive carcinogen. One is that at high exposure levels, we know that inorganic arsenic induces cancer in humans. This is a human carcinogen but the reported levels that are associated with cancer are greater than 150 parts per billion. 150 micrograms per liter. The tumors that are

involved in skin, urinary, bladder, and lung. There have been reports of kidney but Dr. [indiscernible] and his colleagues in studying the Chilean population has shown very explicitly that the changes in the kidney or the pelvis which is really an extension of the bladder, it is the same epithelium, some evidence that liver cancers increase but this is a bit erratic in the literature, more work needs to be done.

With arsenic, the mode of action is fundamentally based on a metabolism which is shown here. This is the classic method of going from inorganic arsenate to arsenate which is then methylated in an oxygenated process that is reduced to the trivalent [indiscernible] all the way to the trimethyl group. It turns out in rodents, mesylate DMA three to TMA 05 quite readily and is a thing of get part of their overall metabolism. In humans, in contrast, we do not do this. The enzyme involved with these metabolism shown by David Thomas at EPA that enzyme does not operate very well in humans with respect to converting DMA to TMA. It is only at extremely high levels of arsenic exposure do you get the trimethyl in the human.

The key for our understanding of this is that inorganic arsenic and arsenic in general has its biological effects by interacting, in trivalent forms, either arsenite, MMA III or DMA III, or arsenate with [indiscernible] to generate these metabolites. Glutathione is present in cells at [indiscernible] concentrations. Arsenic is present in cells at less than 10 [indiscernible] concentration. Above 10 micromolar is lethal. Next slide.

Fundamentally, looking at arsenic carcinogenesis, we have to focus on the trivalent forms and their interaction with specific proteins. The proteins differ from tissue to tissue and importantly, the availability of sulfhydryl and proteins will vary between species. For example, the estrogen receptor in rodents as a free sulfhydryl group that is available for interaction with arsenic whereas that same sulfhydryl group is not present in humans. So for the bladder, with regard to [indiscernible], the area where I have done a lot of research as well as Doug has also done some in this area is that you convert the normal bladder epithelium shown in the upper left-hand corner here by standing electron microscopy which then progresses to cytotoxicity as shown here with the shagginess on the upper right-hand corner and then beginning proliferation in the lower left-hand corner and eventually hyperplastic lesions in the lower right-hand corner. In animals, you rarely get bladder tumors. Reasons that are unclear, but it seems to be related to the metabolism that by six months of age, this process seems to slow down and virtually stop. Next slide.

The same type of things can happen in the various tissues that are involved with arsenic. Whether it is the bladder, the lung or with regard to the skin. So, it involves ingestion of large amounts of the [indiscernible] and at this point, I will focus on inorganic arsenic, degeneration of trivalent forms which react to glutathione and critical proteins which leads to cytotoxicity and cell death, regenerative proliferation and ultimately a low incidence of tumors. This is true for the various tissues that have been identified for arsenic carcinogenesis.

This has been identified in humans, so for the bladder, even though we don't have a good method for detecting low levels of toxicity, when there has been some acute occupational exposures at very high levels, there is clear evidence of urothelial damage which results in hematuria and obvious regeneration. In the lung, there is a fair amount of epidemiology available now showing that chronic bronchitis and bronchiectasis is increased with regards to arsenic exposure and these are precursor lesions that are toxicity and regeneration that if continued overtime will produce lung tumors. And in skin, we have a great deal of evidence from the human, actually much better than from the animals where the classic features of [indiscernible] in humans are the skin changes which include hyper- and hypopigmentation

legions but keratosis has not only increasing keratin but is associated with inflammatory process that leads to increase in proliferation, ultimately dysplastic changes, carcinoma and [indiscernible] disease and basic carcinoma. There is some epidemiology on these early skin changes with regard to arsenic exposure but not much detail. I will come back to that in a minute.

The key is that all of these early changes that are related to cancer are noncancer precursor legions. One can look at the overall risk assessment of arsenic if you have a trivalent [indiscernible] reacting with sulfhydryl groups which is a threshold process. I will go into the details of the basis for that, but these produce noncancer biological effects. If these noncancer biological effects happen to occur in certain epithelia like the skin, the bronchus and the urothelial him, the that they continue overtime are proliferative legions and lead to an increased risk of cancer. There are other toxicities which have been identified with regard to arsenic exposure such as cardiovascular events and possibly diabetes and a few others. These are noncancer toxicities and they are toxicities that are not precursor legions for cancer, but they are important toxicities, nevertheless. One can then focus on the risk assessment for the noncancer biological effects, and if you protect against these, you will protect against the cancer.

So, the overall hypothesis that we are working on is that we have an ingestion of arsenic which is converted to trivalent [indiscernible] and interacts with sulfhydryl groups which is a threshold process and you get a cellular effect. Again, if it is in the epithelial you get cell death, regenerative proliferation, precancer legions and eventually carcinoma. If there in non-epithelial tissues such as islet cells, you get adverse cellular events and noncancer, non-precancerous effects.

One can do a risk assessment on inorganic arsenic for both precancer and cancer identically. Recently, we have put together an approach of a threshold-based risk assessment for arsenic. The idea of a threshold with arsenic carcinogenesis is not new. It was published by EPA more than 20 years ago, and is still being argued about in the literature and in regulatory scenarios but fundamentally the basis for most risk assessments up to date have been based on the identification of adverse events in this instance lung and bladder cancer or skin cancer at high exposure levels and extrapolating utilizing some formula down to low dose exposures.

We looked at it differently. We said okay, based on the mechanistic information that we have, *in vitro* changes and the animal studies we have that we can actually calculate an estimate a human threshold for the effects of inorganic arsenic in humans. This is now in press and *Critical Reviews in Toxicology*. The first author is [indiscernible]. There are a number of us involved with this. Rather than looking at these high dose or high exposure level populations, we said okay, what is in the literature epidemiological he looking at low exposure level populations to see if we can identify a threshold in humans and if that cooperates the calculated estimate that we did based on mechanistic *in vitro* and *in vivo* studies and here is a plot of the various studies that we looked at and you can see that essentially, they all overlap with one saying that there is essentially no effect at these low exposure levels. Low exposure levels, we defined them as less than 150 ppb. Based on these studies we came up with the estimate that the threshold in humans has to be somewhere between 50 and 150 ppb, most likely in the range of 100 to 150 ppb, which matches what we have calculated based on the mechanistic information which is again at a range between 50 and 100 ppb. Next slide.

There also is a number of things looking at the precursor legions and again, the only one that we have, next slide, please. The only one that we have a fair amount of data are the precursor

Bengali area and there has been a number of studies in this population. Go forward one slide. In this West Bengali population which they evaluated the drinking habits, drinking water habits of the population in great detail, a detailed life history of their drinking, they were able to come up with a statement that said that they cannot identify a single case of arsenic and benign skin changes or malignant changes that had an exposure level in the drinking level below 150 micrograms per liter which certainly fits with what we projected. In a survey that I performed a number of years ago for the state of Nebraska because we have exposure levels ranging from two ppb and the drinking water to over 100, I cannot find a single case of [indiscernible] skin changes in talking with all the dermatologist across the state of Nebraska except one case. That happened to be an individual who happens to be taking for reasons that are unknown to everyone solution which is an inorganic arsenic solution. So again, that would fit with the idea that less than 100 micrograms per liter, 100 ppb is not going to lead to these precancerous or cancerous lesions.

[Captioners transitioning]

If we have inorganic arsenic in the drinking water, the mode of action for cancer is cytotoxicity with regeneration. The mode of action involves a threshold and importantly, if we protect the population against these precursor lesions, we will protect the population against cancer. The pressure for humans appears to be between 50 and 150 ppb based on not only calculated mechanistic information but also the epidemiology. This can be extended to non-genotoxic carcinogens in general. The mode of action involves cell proliferation. The precursor lesions lead to cancer over time. It is a necessary step in the process so if we protect against the precursor lesion we protect against cancer. Screening for the precancer events in short-term assays will protect against cancer. We don't need the two-year bioassay to make this estimation.

The next slide shows a list of some references. In the back of each of these references are a number of others. After that slide of references is somewhat knowledgeable of the individuals involved with this recent effort we did. The recent effort to put together this publication in *Critical Reviews in Toxicology* which is an enormous paper, almost 50 pages long. With that I will end, and if there is time for questions, I will be happy to answer them.

Hayes: Thanks, Sam, for an excellent talk. We had two super talks this morning. We have got a couple of minutes if anyone does have a question for Dr. Cohen. Nothing has come up on the screen, so let's go ahead and take our break.

Audience Member: [inaudible]

Hayes: The question is something along this line, Sam. The one hit versus a multiple hit, can the one hit fall under this rubric you are talking about?

Cohen: I don't think there is any strong evidence for a one-hit process in carcinogenesis. If it was a single hit, we would all be born with cancer. Looked back in the original publication by Al [indiscernible], which is based on retinoblastoma, it looked like the kids that inherited the gene had a single hit. The reality was they already had one hit which was coded into their genome and only took a second hit for them to develop the tumors. For people who don't inherit the abnormal retinoblastoma gene, it requires two hits. For the kids that have the gene, the abnormal gene, it still took two hits, it is just they inherent one of the hits. A one-hit process, if you look at any model of carcinogenesis, essentially it would lead to all of us having cancer by the time we are born.

Hayes: Okay, thank you. I don't see any other questions so let's take our break. I would encourage everyone to be back at 10:25 AM Eastern Standard Time. Thanks again, Doug and Sam, for super talks.

Technical Staff: This is a conference coordinator, just a quick note there's network delays of up to 45 seconds so when we change the slides it might take a minute for you to see it on your side.

Hayes: That is helpful to know, thank you very much.

The way you can answer a question would be twofold, when is to type it in under chat or two, if you can raise your hand, we will try to get to you that way. The best way is to go to the chat. If you're on the phone only, I will have to check and get back with you on that. I don't know the answer. We have got 30 minutes to come up with something. We will talk to you guys at 10:25 AM.

Break

Is the Two-Year Rodent Bioassay Needed to Address Carcinogenic Risk for Human Pharmaceuticals?

Frank D. Sistare, Merck & Co Inc., West Point, PA

Hayes: It is time for us to get started. Our next speaker is Frank Sistare from Merck & Company. He is going to share with us his thoughts on is the Two-Year Rodent Bioassay Needed to Address Carcinogenic Risk for Human Pharmaceuticals? Frank, the floor is yours.

Frank D. Sistare: Thank you very much. In terms of the conflict of interest, I am an employee of Merck Sharp & Dohme, which is a subsidiary of Merck & Company out of Kenilworth, New Jersey.

[Inaudible] I am going to talk about two topics essentially. The first one is we have been involved in ICH S1 guidance modification negotiations since 2012. ICH is International Conference on Harmonisation of technical requirements for pharmaceuticals for human use. This is our mechanism in the pharmaceutical world to ensure we are aligned across the world because marketing of pharmaceuticals is a worldwide endeavor. As I mentioned, we have been involved in negotiations since 2012. What we are proposing is to reduce the need for the two-year rat carcinogenic study, not totally eliminate it, but to reduce the need. I will give you a brief historical tour on where we are and how we got here and walk you through some case examples which are poignant which speak to this question of the value of the to your carcinogenic study or at least the perceived value and the differences on the views that exist on that topic.

If adopted, this new ICH S1 guidance was set the stage for flexible future. A vision where we will create demand an opportunity for new toolbox that will optimally leverage what the new guidance will do in terms of opening up those opportunities. My own personal feeling is yes, in the near term, rodent carcinogenicity studies are here to stay for a while, but they more will be judiciously deployed. If we can work together, we will strengthen our position and eliminate the ambiguities that exist and diminish the need of the two-year carcinogenic study for

pharmaceutical assessments over time. We have launched a couple of HESI projects to accelerate that tool to adoption. I think the world is positioned well with regulatory agencies adopting formal mechanisms for new tool qualifications, and we will talk a little bit about that today. I will also give you some case examples.

What I'm going to talk about first is the first topic of where we are now and how did you get here with the ICH S1 guidance modifications. Here is the current state of the world. The current state is we have several guidances which defined carcinogenicity testing requirements for pharmaceuticals. The first one S1 A states any pharmaceutical that is going to be used continuously for at least six months or possibly less than six months at a time but used frequently and intermittently for recurrent conditions, for example antibiotics which you may use for two or three weeks or a month at a time but chronic intermittent use for example. Also cause for concern even if it is going to be used less than six months, this is when you need to use a carcinogenicity study. S1 B is how you will test for carcinogenicity of pharmaceuticals. Since 1997 there was a change made which allowed short- or medium-term *in vivo* broken test systems but the basic testing scheme is one long-term rodent carcinogenicity study plus one short- or medium-term study or you can perform two long-term rodent carcinogenicity studies. S1 C gets into dose selection to make certain that you do it properly. And then S6 is the exciting newer kid on the block which for biotechnology derived pharmaceuticals pressed the dime a bit. How can we really test for somebody's biotechnology derived pharmaceuticals? The acknowledgment was made that rodent carcinogenicity studies are of limited value for these pharmaceuticals and the strategy is based on a weight of evidence approach where information can be sufficient to address carcinogenicity potential without additional studies.

It is the philosophy of S6 which kind of spurred our enthusiasm for bringing that mentality into the chemical world of pharmaceuticals and the small molecules. Back around 2008 or 2009, we launched an effort to data mine the available carcinogenicity data amongst a large group of collaborating pharmaceutical companies, about 14 companies participated. This is not just the drugs that have been approved and marketed but also drugs that were discontinued from development that may have gone through a carcinogenicity study and was discontinued perhaps because of the carcinogenicity study results. The bottom line after all that data mining was from 190 pharmaceutical compounds plus [indiscernible] human carcinogenic chemicals that essentially for 266 chemicals, if there are no histologic risk factors for neoplasia following the conduct of a six-month rat study, if there is no genetic toxicology signal, if there is no evidence of hormonal or pharmacologic perturbation signals, there is no value added in conducting the two-year rat study. Negative predictivity is very good. Overall test sensitivity was 91%. It is not perfect but when you look at the false negatives there is no human relevant [indiscernible] ambiguous results among them as well. These results launched the discussions after bringing the state around to the different regions and different regulatory agencies around the world. These analyses were able to launch discussions to begin if we can modify carcinogenicity testing guidelines while maintaining patient safety and accelerating patient access and projecting the elimination if you look at the data. Estimated around 40% of two-year rat carcinogenicity studies eliminated if you use this approach.

Starting with the end in mind, in the absence of any evidence of a pharmacologic hormonal effect, toxicologic effect whether it is genotoxic or histologic risk factors after six months of continuous testing and acknowledging we do have the mouse, usually it is a transgenic mouse response then a two-year rat bioassay provides very little value in identifying potential carcinogenic risk. You could potentially add in immunology but in terms of immunosuppressant or immune based mechanism you can pick up that signal in a rat study, but the rat doesn't develop tumors as a result of an immune effect, but it is certainly something ultimately you

would bring into a full weight of evidence. In the presence of evidence any findings suggesting potential carcinogenic risk in the two-year rat bioassay may provide information to clarify the level of risk. We will talk a little bit more about that.

As I mentioned, negotiations are in progress to modify current ICH S1 regulatory guidelines and we used the reliance on the two-year rat study. When we got together, we essentially aligned on a concept that with the data we had well very powerful, provocative and supportive, it is a retrospective assessment and oftentimes retrospective studies are not confirmed prospectively.

We did align what we would do is we would launch a prospective study of the concept. We posted a regulatory notice to the ICH website in August 2013 which launched this prospective evaluation period. What this period does it is asks pharmaceutical sponsors to write and complete a carcinogenicity assessment document, and I will describe the content of that document which essentially allows them to assert with a two-year rat bioassay would be helpful for that particular pharmaceutical or whether it would be silly and wasted time. The weight of evidence criteria would establish the different information points needed for drug regulatory agencies to either align or disagree with the sponsor's outcome, a sponsor's prediction. Ultimately the proof would be the result of the final two-year rat carcinogenicity study to match up against the CAD prediction. These CADs had to be written well before the end of the carcinogenicity study. Well before there was any indication of an outcome and we were pretty firm on that. You can see the timeline down below in terms of where we are and where we project to be. The topic was adopted in 2012. The regulatory notice document launching the prospective evaluation period in August 2013, it took two years longer than we anticipated for a critical mass of these carcinogenic assessment documents to come in. It took four years rather than two years, but we did reach that target and now we are essentially waiting for the final study reports to come in.

These are being viewed, obviously the pharmaceutical members cannot review these things, they are being reviewed by the drug regulatory agencies and research is made by an individual sponsor. We anticipate by this November, we will have enough of these category 3 cases, these are the ones essentially arguing for the opportunity for a waiver and will be there by this November and we would hope we could then launch a document for public comment very shortly after, and by 2020, had the entire new addendum adopted.

The next slide describes the weight of evidence development of categorical assignment in the CAD. I will get to the categories next. Knowledge of the intended drug target. The drug target pathway, the pharmacology, any secondary pharmacology, any off-target pharmacology, where's the target distributed? Where is it distributed in rats and humans? Are there homologues of the drug target in the affinity of these homologues? All of the information and published data that exists, are there knockout animals? Is the human genetic data which informs the target? It should be a thorough and complete assessment of the drug target and any related targets. The second one, standard gene toxicity study results. The third is what was launched and histopathic evaluation of chronic repeat dose rat toxicity study, again we want the earlier studies may achieve higher exposures and the product studies as well. Obviously, exposure margins, metabolic profile ensuring we have the metabolite evidence of hormonal perturbation. That was mentioned in the studies and histological durations and any evidence of hormonal perturbation. Immune suppression pops in there.

In our infinite wisdom we added this special section on special studies and endpoints knowing the future is bright and there is going to be new capabilities that will come, whether it is special

stains or genomics, it could be anything. We said this is something that would inform the weight of evidence. Also looking at the nonrodent chronic study and a transgenic mouse study. It is not required for the CAD prediction but certainly can predict. A lot of these things resemble the human element framework and resembled the key characteristics of carcinogens. As was pointed out by Doug, what do you know? What don't you know? What do we think we need to know? That is what we are studying.

Once you have assessed the information about your target like the off target and any evidence from the toxicology study results, you decide whether it is going to be in category one, two or three. Category one is so highly likely to be tumorigenic you labeled it as such and there is no value that would come out of a two-year rat study. It wouldn't add any value. Of the 48 CADs received I think there were two where the sponsor made an assertion to category one, so it is not seen very often, and it is for oncology indications and things like that. The DRAs maybe didn't agree on that assertion as well and felt they would be valuable, at least for one of them.

Category 3, we have categories 3a and 3b. 3b is so clean it is likely not to be tumorigenic in either rats or humans any tier study is not going to add any value and we still talk about keeping the mouse and we do realize a transgenic mouse probably comes with the fault as time goes on. Category three A is something we have heard about it today's discussions. It may be likely to be tumorigenic in rats but it's not going to be tumorigenic in humans because we know if this mechanism and we know it is irrelevant to humans or we know at the doses we are going to see in the rat is not going to be relevant to the humans at the doses we are going to be giving so that can be category 3a.

Category two is the one where there is enough uncertainty around tumorigenic potential for humans based on all the information that the rodent carcinogenic study is likely to add value to human risk assessment. Of the 48 CAD that were submitted, 24 of them are being categorized as category 3a. The sponsor asserts it to be category three at least one of the DRAs. We have five voting DRAs right now. It is another problem if we can't get all DRAs around the world to agree that we do at least for this purpose a if there is at least one neutral regulatory agency we will call that a category three. We wanted to make certain during this process we didn't get any category threes that would be a failure.

The next slide is the inventory that describes the makeup of the category threes we saw. Sponsors asserted 34 of the compounds as being category 3. The regulatory agencies agreed unanimously on 14 of them. For another 10, one or more DRAs aligned with the sponsor but there is a split decision. What about the other 14? Both the sponsor and the DRAs agreed for at least 12 of them, that's important. We have sponsors and regulators agreeing this two-year study is going to add some value on 12 of these 48 compounds. That's 25% of the time. 50% of the time it is probably not going to add a value and then is going to be disagreement on the other 25% so that is where we landed there. We have got 24 final study reports so far that have been reviewed by DRAs and 12 of them fall into category 3. We have had excellent discussions in our negotiations in terms of why. We have got that, and I'm going to talk a little bit about that with you.

First of all, big buckets. There are three root cause buckets of discordance. One is the data in the CAD is, is viewed as insufficient. Either the sponsor didn't do a complete job or at this point in time sponsors would generally not generate data unless they really need to, but every talks signal the bricks may not have been addressed and that may be an artifact of the state we are in. The second is first in class molecules. New target, new biology a new chemistry is viewed with a much higher level of uncertainty by regulatory agencies, and that is something we are

going to need to deal with. A third bucket is the concept that it is likely going to be negative but there is to value of an opportunity to pass up. And -6 month weight of evidence argument is not going to cut it. They want to see more. What do you know? What don't you know? What do you need to know? The sponsor is one opinion on that and regulators may have a slightly different opinion.

Let's talk about some of the category twos where sponsors and regulators agreed there is some value of conducting the two-year rat carcinogenicity study. These are high level and no disclosure of anything proprietary and I'm going to talk about a case which is a Merck case. Case one, here is a first in class molecule for a serious indication but maybe not for a debilitating life-threatening disease. If you look at the literature it indicates there is a likely significant potential for possible tumorigenic target-based mechanism, however, it is negative for gene talks chronic study and immune type of facts. It was agreed there was probably potential in coming up with a carcinogenic study result.

Second, the first in class convention a debilitating degenerative disease. The literature review indicates minimal potential for possible tumorigenic target-based mechanism, however, there is hypertrophy without any mechanistic explanation. If it doesn't turn into a tumor it is one last thing I sponsor would need to open up. At this point in time it is not something that is done. Probably the easiest of the group to deal with.

Case three is the first in class for a viral infectious disease so it is not after a mammalian target. There is really no mechanistic concerns. It is negative for endocrine's and his style but in a short-term *in vivo* detox study, we do see a signal of high exposures and the question is what if I dosed this for two years in a rat and I have a legitimate mechanism, is this going to trickle down to lower dose levels? Again, the sponsor and regulator agreed there is probably some value.

Now I'm going to describe another scenario, and this is something Merck has seen. This is what I call a nonstarter case. It doesn't make a category because we as a company decided it is not worth the risk. It is a new target first in class for a serious but not life-threatening disease. The literature review indicates there is significant potential for a possible target base and it would be considered human relevant tumorigenic mechanism. I'm going to show you a few slides ahead where it is in the top 40 list of the cancer driver gene pathways. We're talking about pharmacologic the manipulating something. When you look at the literature, they go to the trouble of making conditional knockout mouse because the map out did show tumors and you go to the trouble and you see tumors. They started developing the drugs and the tumors are expected in rodents and humans but it was a target or it [indiscernible].

The next slide is a detailed slide. I think the slides are going to be made available, but it captures the thinking I just described. The first two rows of comments are nothing new. Are they going into old people or children? Is the model a good one? Does it express receptors or any value? When you get into three and four you get into this questions you ask yourself about the novelty of the target and how convincing are the data? Whether there is risk or isn't risk and the last is the specific drug associated data the sponsor has been able to provide. A lot of these are reminiscent of key characteristics of carcinogens.

As I mentioned this new ICH S1 guidance if adopted will set the stage for a flexible future, but it also creates an opportunity for new tools. I'm going to go through some of those opportunities and what we are doing about it and again provide you with some examples. On the next slide when we think about the problems that are going to limit implementation, there

are two key problems and opportunities. One is the false positive question. When we did our data assessment from the farmer perspective, about half the pharmaceuticals in a six-month rat study they will present with some histologic neoplasia, but they will not turn into tumors. They still need to be explained [indiscernible] require explanation. What is the mechanism underlying these histologic risk factors? We feel a signature approach can be used to identify some of the common mechanisms. Reactive hyperplasia following repeat tissue injury. DNA damaging and genotoxicity. We have launched a couple of projects under HESI to align the world on the use of these, so we understand what we are dealing with. We have a couple of projects launched. We are meeting face-to-face with an assembled group for the first time. We have a genomic signature technical group. We have a gene toxicity group which is examining an exciting new technology of the next-gen sequencing that essentially captures the second problem.

The second problem is the first in class pharmaceuticals. They are receiving lower levels of alignment between DRA and sponsors. DRAs are usually cautious even when there is no risk factors and there may be nothing in the literature that suggests a concern. And negative TRS study is being hewed [indiscernible]. Is that the way it is going to be forever? I hope not. I am hopeful if we follow the logic of virology some of these additional approaches can be used to embellish the outcome of these negative studies. Experts have asserted for many years there is limited modes of action that exist for carcinogenesis. There is generally three categories. The hypothesis is there is this new capability next-gen sequencing together with these signatures can inform when a drug may or may not be driving a growth advantage mutation associated with cell survival, cell fate and we can pull together a lot of study data and a lot of resources and samples that exist and a lot of unique pharmaceuticals that can blow this thing open.

The next slide is a placeholder that says I'm going to talk about the genomics aspect. If you get a negative scheme you are feeling good but there is a significant false positive rate, so we need to explain the positives and what is driving those changes. What is nice is there is a lot of nice parallel effort. This adverse outcome pathway targets of interest align really well and there is a lot of work. I probably need to update this, all the efforts underway to align what we can do and how we can get the pathway guidelines going and all these mechanisms.

If you take a snapshot of our own experience here at Merck, we have been looking at short-term studies and doing targeted gene expression analysis for number of years. This is a snapshot of pharmaceuticals that have been nominated for single candidate selection. You see a significant amount of perturbation of pathways. This is still something we see in the chemicals we bring to bear. These are going to surface as we go into chronic studies. Again, the dose would define the poison in the dose is going into people and animals, are they relevant to the doses we are testing upfront? That takes time for that to get sorted out, but this is a snapshot. 20% of compounds present with the significant AhR score. 4% are really strong AhR agonists. We have drugs that are hitting receptors [indiscernible]. They could come back to haunt us in the form of the study that again would need to be explained. Our own labs here at Merck and the lab the EPA and leveraging our internal data and publicly available databases, if you look to the compounds where there is overlap between the doses cannot did [indiscernible] you can account for a high percentage of the compounds that have labels in the PDR for these kinds of tumor outcomes.

A quick example of first in class molecule could be considered human relevant off target risk factor and that is AhR activation. A published example that typifies something we saw here at Merck, this was published by the GlaxoSmithKline people where they saw tens of thousands of

fold inductions of [indiscernible] which indicates a strong and potent AhR agonist. They say were going to be giving a pretty potent [indiscernible]. They found it was very strong in human and the purple line is the molecule in the drug class and say this is a much better molecule to go forward with. You can go into the literature. AstraZeneca presents a similar situation. Pfizer indicates they had chemist that were coming down with [indiscernible] because they were dealing with drugs that were strong agonists. We had a situation where we had to deal with this.

A publication came out in 2007 which surveyed the world of pharmaceuticals, it says there is a lot of AhR agonists. 239 compounds had some activity. You need to have a certain threshold and magnitude and it needs to be sustained for the AhR agonist to be a tumorigenic risk factor. The dose defines the poison, the dose are going to test in the study is going to tell you what you are likely to see. What happens in the rat carco study is going to go into the drug label so it's important what is going to happen and the exposure margins in the absence of any definitive human data.

What we did was the kind of thing we are going to do in the HESI project and that is you need to resolve ambiguity. We live in an ambiguous world and ambiguity is going to exist and we need to understand when the dose and exposure is knocking on the door of danger and when in my below the threshold? A lot of these are typical of the worst actors. It is not sustained [indiscernible] below any threshold of concern. If you look at a non-tumorigenic dose and compare it to tumorigenic dose of [indiscernible]

Hayes: Frank, you got two minutes to wrap up.

Sistare: The next slide shows the Merck experience. We see every once in a while the upper right-hand bucket the hair concern, we see AhR so that is something we need to deal with. If we were blind to this and processing to development, this could come back to haunt us. The next problem is around first in class and asking the question of can error corrected next-gen sequencing help us?

What is happening in this world around us is the world is leveraging comprehensive human DNA sequencing data. We can leverage that to also understand target native potential for pharmaceutical carcinogenicity. Again, the three buckets of pathways that are essentially associate with tumorigenesis [indiscernible] very highly cited publication which relate to the key characteristics of carcinogenesis and ways to approach that and how you fight cancer. We can leverage that work.

All of the sequencing data that has been accumulated on human tumors, there are driver genes and passenger genes. In 2013 there were about [indiscernible]. A typical tumor contains two to eight driver gene mutations. There may be passenger gene mutations. If you look at the lung tumors from smokers, you will see about 200 mutations. For non-smokers, 10 to 20. They're the ones doing the work and the ones coming along for the ride.

The next slide, they're databases that have this human sequencing data. The next slide, this is an example of a website which shows you the cancer driver genes. The sizes indicate the frequency. The next is the frequency of the catalog of human mutating cancer driver genes. Here is the top 40. Next is the specific mutations you can get out of this information.

The bottom line is all tumorigens are going to cause, encourage or allow driver gene mutations that convert, all roads lead to Rome [indiscernible] whether it is affecting the immune system.

Ultimately if we can survey these things, this is where we are going to win this war. Ultrasensitive sequencing is a reality and is being applied to diagnose and catch mutations earlier. This is an example of that [indiscernible].

Hayes: Frank, for us to keep on time I'm going to have to cut you off.

Sistare: There was one more slide and there is a plug for Barbara Parsons' publication recently who has been working this field for many years and is an advocate and this is indicating we are on the right path. Thank you.

A Weight of Evidence Approach to Cancer Assessment

Alan R. Boobis, Imperial College, London, UK

Hayes: Thank you for a very good talk. I would like to remind everyone if they would to submit their questions for the panel which will be coming up after our last talk. I'm delighted to introduce Alan Boobis from the Imperial College of London who is going to close this out with A Weight of Evidence Approach to Cancer Assessment.

Betty Eidemiller: We are just making a switch and will be on the way in a minute.

Alan R. Boobis: I appreciate the invitation from the FDA and SOT to participate in this workshop. I'm going to pick up from Doug and Sam's talks to illustrate some of the concepts they presented on using emerging knowledge of mode of action and weight of evidence in reaching a conclusion on whether or not there is a concern of carcinogenesis from chemicals. I should emphasize that the overall objective is not to do a risk assessment for cancer, but to determine whether a chemical exposure is at a level that is going to be sufficient to protect human health and no concern for any effects in humans.

I have no conflicts of interest in, in the interest of transparency, however, I have disclosed relevant other interests and none of these are financial.

This is part of the concern many of us have. When you run a high-dose cancer bioassay, you are using the small amount of information available. You simply dose rats and/or mice usually in the high exposure level and look for the presence of tumors after two years and of course any time after the two years you conclude this study. This completely ignores the nature of the dose-response relationship and whether it has any biological threshold, whether there are any important differences between the experimental model and human populations other than the particular circumstance where you might conclude there is a qualitative difference that lets you exclude relevance. If the tumors are relevant, we don't take into account species and sensitivity either in kinetics or dynamics. Nor of course any consideration of the actual exposure level of populations of concern, so there is no risk characterization performed. Unlike pharmaceuticals where the objective is to treat patients with the biologic effects which may or may not be linked to a potential target of interest, for carcinogenesis it is to find a level below which we think there is no risk of adverse health effects so there is an appreciable difference in the importance of exposure assessments where we can argue if we are above exposure level of concern we just want to improve the pesticides.

Just to reiterate some of the issues, that the cancer bioassay, I think it is fair to say if we were to start to say [indiscernible] giving animals these compounds we probably wouldn't do it quite

the way we have been doing it for the last 50 years. I guess it is over 90% of all studies are conducted in rats and/or mice. We have to be different in specificity for us to use a non-rodent species. By and large always use rats and mice for reasons that are obvious. A nominal lifetime which is now two years, and high doses because we need to facilitate the identification of the hazard regardless of the potential of human exposure, and of course the limit dose is determined by the possibility of adverse effects in animals that the [indiscernible]. Hazard identification is based on tumors. We do a pathological evaluation of the tissues and reclassify them to tumor pathology basis [indiscernible]. What is the parent now minute chemicals cause carcinogenicity other than by directly interacting with DNA and secondary to the primary toxicity. The relevance of tumors produced at high toxic doses of a chemical to human health risk is highly questionable. If the tumors are rising secondary to the toxicity occurring and that is toxicity is only occurring [indiscernible] human exposure. We know some mechanisms of carcinogenicity in rodents are of little or no relevance to humans because of fundamental difference in biology. Tumors in the male rat kidney induced by precipitation of [indiscernible] will have no relevance to humans.

This builds upon describing the idea that there are several limited ways in which you can beat the point at which there is total expansion of the population of transformed cells to emerge as a tumor. When this you can take the DNA by binding to it either a chemical or reactive metabolite causing gene mutations and critical target genes and if the cells continue to survive, they require additional mutations which eventually leads [indiscernible]. Mutation is a spontaneous process. As you heard it to stimulate the proliferation of cells either direct, for example through hormone or indirectly by causing the destruction of some of the cells resulting in a regenerate of proliferation of the stem cells to repopulate the organ with adult mature cells, you will see this is prolonged and over a period of time the emergence of tumors and other possibilities include repair which simply exacerbates the ongoing process or immunosuppression which means you prevent the removal of some of the mutated cells that are otherwise destroyed.

I want to illustrate application using a case study and this extends the philosophy Doug and Sam were providing that if we got sufficient information from shorter-term studies, we don't really need to go to a long-term cancer study to protect human health. This was a question put to the [indiscernible] in 2011 and that dates will become important as we progress in this case study. This is one of the flame retardants, TBBPA that is a relatively high molecular weight. [Indiscernible] used primarily as a reactive flame retardant. It is not going to, easily produce is the flame retardant which means there is the potential [indiscernible] into the environment and into the food. The question they were asked is what is your opinion as to the potential risk of consumers of food that contain the contaminant TBBPA? I should say now we have available a database which did not include any studies longer than 90 days, so we were trying to reach a solution in the absence of a carcinogenicity study.

In a 90-day study, we saw some indications of effects on the liver which were decreased [indiscernible]. There were slight changes in circulating oxygen levels at the highest dose. We also had the two-generation study in rats. It was a decrease in T4 at 100 mg [indiscernible]. In the developmental toxicity study in rats, females showed a decrease at 100 mg per K but there is no evidence in the multi-generation study in the developmental toxicity study of any reproductive or developmental toxicity. This would suggest there is no endocrine activity at lower doses. We were seeing no changes in the developmental landmarks or reproductive performance of the animals.

In terms of immunotoxicity there was side effects of host immunity in mice treated with 1700s make per K per month. There was no effect on the immunization response to the blood cells in rats up to 3000 g per kilo per day for 28 days. Any immunosuppression was marginal.

We looked in more detail at the studies and trying to explore the effects of TBBPA in the liver to get some mechanistic insight. In the 90-day study there was a decrease of bilirubin at the highest is in males and the females was [indiscernible] per day. There was some increase in [indiscernible] levels in females. [Indiscernible]. There was no other effect on the semen parameters of liver function and there were no histopathological changes in the liver, necrosis or [indiscernible]. Obviously, the question was [indiscernible] the only study is a 28-day study and there were no [indiscernible] on the prototypic [indiscernible]. In mice treated with 1% over the period of gestation, the [indiscernible] showed an increase in liver weight and there was evidence of necrosis and [indiscernible] in the liver. The 14-day said in mice there was some increase in liver weight of 1400 and some evidence of histopathological changes. The picture is 300 megs per K we are beginning to see the effects in mice and some at higher doses we might see the effects in male rats. That they strongly indicative of what you would expect.

Obviously, one was concerned about the possibility of genotoxicity. We had a good range of tests *in vitro* but not *in vivo*. *In vitro* in salmonella strains either with or without the standard metabolic activating system there was no evidence of genotoxicity and up to 10 milligrams and tested at very high doses. This substance is not particularly toxic to these organisms. In a chromosomal aberration test, there was no evidence of [indiscernible] up to 75 µg per ml with or without metabolic activation. [Indiscernible]. There was also [indiscernible] and there was no indication of any change. Our conclusion from the panel was TBBPA does not represent any risk of genotoxicity.

The panel had to reach a conclusion as to whether we would say we can't give any advice because we have no adequate information on the carcinogen potential of this compound will could be based on the information we had from the shorter-term studies reach some advice to the risk managers on the safety of contaminant levels in the typical European [indiscernible]. Based on the absence there were no [indiscernible] no evidence of immunosuppression except possibly high doses and no evidence of reproductive or developmental toxicity, the panel concluded the weight of evidence supported the conclusion there are no indications TBBPA might be carcinogenic, and I should've said this is that tumor exposure levels. As a reference point, the critical effect upon which our [indiscernible] was based was hormone levels in rats [indiscernible] for effects in females and this was used to calculate margins of exposure against the estimated exposure of European populations. This was in 2011. I have no information we had reached a conclusion that based on the information we had there was no concern about carcinogenic risk.

In the meantime, supplicant to the publication the NTP published a conclusion of a lifetime study of this compound in rats and mice. This was essentially a prospective evaluation along the lines Sam was describing where we made the call before we had the results of the cancer study. In this study [indiscernible] a full NTP study where they did short-term studies, 90 days and mice in the two-year study in rats and mice. In rats they confirmed there was a decrease in [indiscernible] so this is a consistent finding. There were some increased liver weights but no changes in the hepatic histopathology. There is probably some sort of [indiscernible] going on. In the mice the liver weights were increased and there were some effects in the kidney. In the cancer study, you know [indiscernible] they found clear evidence of carcinogenic effect in females based on increased incidence of uterine epithelia tremors which were predominantly

uterine adenocarcinoma. In the two-year study in mice in males, some evidence of carcinogenicity based on the increased incidence of the hepatoblastoma.

You'll see there was evidence for precursor lesion in the endometrium. Maybe there was a marginal nonsignificant change. There was an increase in adenocarcinoma. A significant 5% level. And there was something possibly going on with this malignant mixed Mullerian tumor. So there appeared to be, there was an increase in adenocarcinoma which was preceded by the effects on the endometrium and appear to be high dose, long-term effect only seen in the longer-term studies. Just to remind you by which we judge the protection of the exposed population was 16 mg per kg per day, effect level for the adenocarcinomas in this study.

In the male mouse liver, they saw evidence of 250 and 500. Some evidence of the hepatocellular adenoma. There was some suggestion of an increase of hepatoblastoma. 5%. The higher dose, 500. And it was not significant by a poly-3 train test. Poly 0.065. That's why there was some evidence. If you combine all three types, considered to form a spectrum of carcinogenesis, you'll see there's no indication of increase across those groups. There was some evidence and arguably 500 is the level but even with 250. Even if we have this, I don't think it would have made any difference at all to the risk characterization that the panel conducted on its conclusions that with the critical BMDL, the exposed population was adequately protected. Skip this one. Next one, please.

So, this is to build more on the mode of action. We didn't have a mode of action for the risk assessment. Here's an example where we did use the mode of action. This is for a pesticide. This is work of the pesticide residues. 2016, I think. It builds upon this concept of a mode of action which you've already heard described today. Originally this was used to exclude the human relevance of tumors seen in experimental animals like rats or mice, but the recognition, the knowledge of the mode of action gives a lot of useful information on the relative risk to humans. In addition to that, as you heard from Frank was that we traditionally used mode of action, we've had a observation of a tumor then tried to construct a mode of action. You could think about where you actually measure the key advantage *in vivo* or *in vitro* and then predict whether you've got a likely percentage.

This is a case study on chlorothalonil. This glutathione conjugate is a substrate for, is then taken up. Sorry. It's taken up in the kidney and in the kidney it's subject to further metabolism by cleavage of the [indiscernible] in this case the renal proximal tubal cells. This kills the cells and as a consequence, renal cell proliferation. Which allows, eventually, if this is prolonged, adenomas and carcinomas.

So, the question is do we need to protect your health by considering this a carcinogen? Or can we think about using the obligatory renal toxicity as the basis of protecting human health? We look at the evidence for the toxicity and the rat. There's increased renal weight in the weight in the 28-day study with the lowest observed affected level 80. There's lots of changes in renal parameters like increased BUN, increased renal weight. In another 90-day study, where they dropped the levels, they found the effect level for hyperplasia of the epithelium of 10 and increased renal weight of 1.5. The 1.5 was the lowest NOAEL. The two-year study, they found increased renal weight of 3.8. Hyperplasia of the epithelium of proximal convoluted tubules at 1.8. The 90-day study was numerically the same as the indication for renal toxicity in a two-year study. Next slide, please.

After two years in these rats, adenoma and carcinomas. Started at 40, went up to 75. There was evidence in males and females of an increased incidence in the lowest tested at 40. The

second study started with much lower doses. Absolutely no indication of a tumor response up to 3.8 in males or up to 15 in females. Then we begin to see evidence of tumors in the males at 15 and the females at 175. And we look at those studies together, it's a consistent picture of an overall level for tumors of 3.8 in the males. The information of the kidney tumors were the direct result of prolonged renal cytotoxicity. The renal toxicity in the two-year study using a safety factor of 100, so we come to the same place if we use the results of the study. Provides a margin of 200 for the tumors in male rats. We could have gone further if we had the information but we didn't have it, which is that based on this mode of action, humans are less sensitive because of the relativity of the enzyme, but it's certainly not possible to dismiss relevance to humans on quantitative grounds, because we could not quantify the difference because we didn't have the data. It's very likely that the ADI, based on renal toxicity, consequence of the formation of the bioderivative would be conservative and therefore would be conservative for protecting against any tumor risk as well because that is a consequence of the progression of the proliferated cells. Next slide.

So, to conclude, cancer is often secondary to primary toxicity through a nongenotoxic mode of action. The key events involved in this carcinogenic response are often reversible up to a certain point. And will occur sometime before the emergence of the tumor. Usually a lower dosage. So, a risk-based approach based on the overall weight of evidence would be more than adequate to prevent any risk of cancer, avoid the unnecessary attrition and restrictions of otherwise useful substances. Because I think often what we miss in this is that chemicals are being developed, not just trying to poison people. For a particular purpose. It doesn't do anybody any benefit if we eliminate otherwise useful molecules on known scientific route, so I think it's important for the risk assessor to provide the risk manager with as accurate information as possible to allow a judgment to be made. Next slide is just a reference list.

Roundtable Discussion: How Can the Rodent Bioassay Evolve to Meets the Need of Predictive Toxicology?

Moderator: A. Wallace Hayes, University of South Florida and Michigan State University, Temple Terrace, FL

Panelists: Todd Bourcier, US FDA, Silver Spring, MD; and Janet Zang, US FDA, College Park, MD

All Speakers

Hayes: Thank you very much, Alan, for another very, very good presentation. I want to thank our four speakers for their input. We really have got a lot to digest and think about as a result of the speakers. We're going to now have our panel discussion. And in addition to our four speakers, we have three individuals that will also be on the panel. Warren Casey from the National Institute of Environmental Health Sciences. Janet Zang and Todd Bourcier, both from the Food and Drug Administration. So, if you have questions, you can go ahead and type them in, and we will do our best to get to them in the next 25 or 30 minutes. Why don't we start with our last speaker? Alan, here's a question. Was the BMDL of 16 mg/kg from a 90-day study?

Boobis: No. It was in fact from the two-year study. From the 98 study.

Hayes: Okay. Thank you very much. Frank, here's a question for you. Are there efforts to waive the mouse study, instead of using transgenic models?

Sistare: Yeah, so when it makes sense that there's going to be little value in conducting a mouse, then yeah, that situation would allow for a waiver. But that's not our mainstream thinking right now. There's some discussion going on about how to deal with the mouse, but the initial proposal is that there will be a mouse. It will be transgenic, and the discussion is around the waiver of the rat. Again, in some situations, it may make no sense at all to conduct the mouse as well, and we'll deal with how to deal with that. I'd give Todd a chance to maybe speak to that as well. Todd, you want to comment on that?

Hayes: Todd, are you there? No response. Todd, if you do come on, just jump in, and we'll let you respond to that question. Doug, here's a question for you. Any other effort beyond using read across approaches for agrochemicals? Do we have estimate how many existing agrochemicals fit into certain chemical classes? Why don't you respond to the first question? Any other efforts beyond read across.

Wolf: Well, read across is just one piece of information. Yes, there's a number of efforts looking at alternative approaches. I think to bring in some of what Dr. Sistare was talking about, using benchmark dose modeling as Dr. Boobis was talking about. But in shorter term studies, they do predict virtually the same point of departure as the chronic studies. So, there are a lot of other alternatives to using shorter term studies and other data streams than just read across. I think the issue with read across in the context of waivers is what the regulatory folks are talking about is what do we already know about that class of chemistry? But the other component of read across which we can't forget, particularly in environmental contaminants, is how the product is used. So therefore, what's the exposure? And is there something we can learn about the potential human exposure from other products that are used similarly? And I think that's also a very important component for those products that are not intentionally dosed into people like pharmaceuticals. So, there's a fundamental difference between the pharmaceuticals and some of the concerns from chronic exposure because of the much higher doses you're exposed to in pharmaceuticals than in environmental contaminants and agricultural products that the human exposure is very, very low.

Hayes: Thank you. Second question, Doug, do we have estimates as to how many existing agrochemicals fit into certain chemical classes?

Wolf: Yeah, there's actually some nice posters out there that you can find, fungicides, herbicides, insecticides that are registered in different areas that cluster them based on their chemical class. So, some of them, very few of them are unique. Many of them have functional changes on the molecule that may change their physical chemical properties a little bit, but they tend to be in a finite number of chemical classes.

Hayes: Can you share a website with the audience that they might turn to?

Wolf: I'll try to find it and I'll send it to Betty, and she can add that to the various links that will show up on the future website.

Hayes: Thanks. One additional question for you, Doug, what if a new chemical in a development could not use this approach for a waiver purpose? How do you handle that?

Wolf: So, I think one of the other things that Dr. Sistare implied but didn't actually talk about is the importance of direct interaction communication with all the different parties, including the regulatory agencies. And that is absolutely critical. So, if it's a brand new class of chemistry, and some of the example that Professor Boobis showed, we didn't know the answer to this

cancer bioassay but we went ahead. Having that discussion and trying to understand what do we know, what do we need to know, you may not, if it's first in class and you don't know enough, then that's a good rational justification for doing the cancer assay. If you know enough or if you understand the exposure sufficiently that a cancer bio won't add to the decision, then that's a decision you make collaboratively with the regulatory agency. We're very fortunate in the United States and North America to be able to communicate very directly with our regulatory agencies and U.S. and Canada. It's true of several other countries around the world. Some countries, it's very challenging. So shows of you who are international listeners, I think that becomes a challenge to move away from doing some of these studies because having that direct communication and discussion is critical to get to where the pharmaceutical companies have gotten to of really being able to identify specifically when it's valuable to do cancer bioassays and when it won't add to the database sufficiently.

Hayes: Thank you. The next question, I'm going to start out with Sam and then if anybody else wants to jump in. And the question, Sam, is what level and duration of proliferation can be interpreted as a cancer risk? Is a short term burst of proliferation in a two-week study that does not show up in a four-week or longer duration study, is that still a risk? Sam, are you still there? Sam must not be with us, so I'll ask Alan if he wants to respond to that and then anybody else.

Boobis: The answer is maybe. It depends. The problem I think is the measurement of proliferation. We don't have great methods sometimes to measure low levels of sustained proliferation. I would argue if you get a short-term effective proliferation, it stops. If that's a true cessation, I don't think the cancer has increased. I think if it's a prolonged low-level increase of proliferation, even if it's not measurable, then it's likely there will be an increase in cancer risk.

Hayes: Doug or Frank, do you have a comment?

Sistare: The only thing I'd add is, yeah, I think Alan really hit the nail on the head. It depends on the definition of proliferation. How confident are we? Are we certain there is no proliferation after that two-week point? I would say if you have a chronic study and you have no histologic evidence of any sort of effects of a sustained proliferation, I think it's unlikely you have a risk. That would be my perspective. It just depends on how confident are you at that four-week time point when you're looking at something that you don't have a proliferative effect going on. The only other thing I'd say, I know Abby Jacobs, when she was at the FDA, looked at 13-week studies and was not as informative as the chronic study with respect to resolving this kind of thing where you can see something in a short term study but a longer term study it disappears, it tends generally not to be a risk. It's a tough one without seeing actual data.

Wolf: Sam, are you there?

Todd Bourcier: This is Todd. I'll comment later. You can go ahead, Doug.

Wolf: I was just going to say that that question comes out of the concept of mitogens, you see that initial burst of proliferation, but don't forget what's happening there is you do get increased proliferation, but you get increased numbers of hepatocytes so the population of cells at risk is increased. So, while when you do cell proliferation, it comes back as a percent over time for these mitogenic chemicals, you're driving increase numbers of cells. That's why we started looking at this population-based model for carcinogenesis. The other critical part is it's not just the initial exposure of burst of proliferation, but also continued exposure, continue driving that response over time and so it's both sufficient dose and sufficient time even for the mitogenic

chemicals where you get the burst early and it goes away. You still have more cells in the liver that are proliferating.

Cohen: Wally, this is Sam. Can you hear me? Something happened to my computer and cut the whole thing off so I'm now back on my phone. Doug has hit the point exactly. I think that's why looking only at the 13-week study is going to miss some things if you only focus on labeling index or other things. You really need a combination of the one-week, four-week, and 13-week evaluations for cell proliferation. If you combine those, not only the labeling index issue but also look at just histology for evidence of hyperplasia, organ weights for increase in organ weight, as well as serum chemistries can be done and here's where I think genomics will come in because they're as sensitive as the labeling index and in some instances even better. I think detecting the labeling index is important, but we have a lot more markers than looking at a PC&E or Ki-67 labeling index.

Hayes: Okay. Great. Any other comments? I have more of a comment than a question. Somebody wrote in, confused by several presenters suggesting the need for exposure assessment to be included in top risk assessment is a new concept. Exposure assessment has been a significant component of regulatory risk assessment and decision-making for decades. Anybody want to respond to that comment? I know who it's from and it came out of the FDA, but I won't divulge the name. Anybody want to comment?

Wolf: I don't think we're saying it's a new concept. What we're trying to suggest is that the change that's proposed here is looking at exposure first. Drugs for pharmaceuticals and even food additives, you know what the exposure is. For environmental contaminants and crop protection products, it's a little different. A lot of times we do a lot of the animal testing and worry about exposure later. When you do the risk assessment. Whereas we're proposing, consider exposure first before you start looking at all the potential hazard concerns. So, it's not, I agree with you 100%. It's not a new concept. Risk assessment is based on exposure and hazard, but it's what order do you do the evaluation strategy in?

Janet Zang: This is Janet Zang.

Hayes: We can hear you, Janet.

Zang: I just want to add a couple points from the perspective of food additives.

Hayes: Can you speak up just a little bit?

Zang: Okay. Let me try. Hello? Is it better?

Hayes: Yes.

Zang: Okay. I just want to add a couple points from the food safety regulatory side. Traditionally and in many cases, we have a tendency, or we're forced to make a binary decision of carcinogen versus noncarcinogen. But from today's talk, I think a message delivered to all the audience is carcinogenesis is not binary. Because it's involved in many steps and many factors, it's more of a probability. And that's why we have to think more about exposure, without thinking about exposure or problem formation at first, we will fall back into making decisions of yes or no, and so-called the Delaney clause that many of us have heard of. That's why I think the emphasize of exposure is just making sense.

Hayes: Any comment or response to the comment just made? Sam, here's a question. How practical is it to determine subtle cell proliferation in tissues for a new chemical?

Cohen: As I tried to point out in my talk, it would be a waste of our time to do it for all tissues because for many of the rodent tissues, extrapolation for cancer risk is negligible to begin with. Endocrine tissues. You can do a sample of endocrine risk in other ways. Otherwise I think for the organs where it's doable, if you combine multiple time points as well as multiple doses, it's very sensitive. And doable. And I think adding genomics going into the future will make it more sensitive.

Hayes: Any other comment from the panel? Another question. Most of the approaches discussed here have addressed false positives from rodent bioassays but not false negatives. Chemicals which might cause human malignancies for which rodents are poor models such as colon carcinoma, which is of obvious interest to the FDA's Center for Foods. Dr. Cohen pointed out rodent bladder epithelium did not prove to be a good model of the effects of inorganic arsenic and metabolized in humans. How can we use our new knowledge of mechanisms to evaluate bladder cancer risk when we do not have epidemiological data as we did for arsenic?

Cohen: If I can address that, wally, this is Sam. I did not say that the bladder model is not good in picking up proliferation. It's just not good for picking up tumors. In fact, if you look just at proliferation as a predictor and looking at mode of action, looking at the short-term studies is much more sensitive and predictive than the two-year bioassay. So, the two most notable exceptions is cigarette smoking and inorganic arsenic, which are almost always negative and lots of reasons. Both of them are picked up in the 13-week bioassay or a four-week bioassay. Then you can work out mechanisms and all that kind of stuff with metabolism without waiting for a two-year bioassay to tell you in the rodents, they don't get tumors from those substances and they're well known to be human carcinogens. I'm not aware of any known human chemical carcinogen that isn't picked up in a short-term bioassay, where some of them, like cigarette smoking, inorganic arsenic, are negative in the two-year bioassay.

Hayes: Thanks, Sam. Any other comments? Another question deals with interindividual variability. How does this effect the threshold, and how should it be addressed?

Boobis: So, we do address already where we accept that the population varies [indiscernible]. If you go follow the rationale that the carcinogenic effects we've been talking about today are secondary to toxicity, the primary toxicity, the variability in the response to the toxicity will be a primary determinant of variability in carcinogenic effects. It would only show additional variability to the extent some people will be resistant. That is not everybody who shows the toxicity will develop tumors. But nobody who doesn't develop toxicity will develop the tumors. As a consequence, if we allow for the variability, which we do already, in the primary response in our health-based volumes, we'll protect against variability and tumorigenic response to this class of carcinogen in my view.

Hayes: Thanks, Alan. Any other comments from the panel? Got a question for Frank. How do the classic mechanisms of drugs submitted during the CAD process compare to the classic mechanisms introduced in the 2011 retrospective analysis?

Sistare: That's a hard question to answer. If it the question is--

Hayes: That's why we have experts like you here.

Sistare: So, let's think about the target. The targets are very different obviously from 2011 backwards, we were drugging very different targets than we are now. So, the intended targets are very different. So, if a tumorigenic mechanism is associated with a drug target, it's going to be different. But I will say that, again, I'm going to ask Todd to chime in here. Of the 48 CADs we've received, of the 24 final study reports we received, there's really no flaming surprises there. You can raise your eyebrow maybe once or twice, hey, what's going on here? But it wasn't any surprise that didn't come out of any of the other data that indicates that there might be some risk here, that kind of a thing. Are the mechanisms that are responsible for driving the tumors in the 2011 database that we're seeing largely a lot of off target stuff, is that going to be the same when we do see tumors in some of these other reports? It's too early to tell. I don't have an in-depth view of, for example, some of those category 2s that may show a tumor, what's driving them? It's too early for me to comment on that. But again, Todd has a much better view of the data, I only get high level reports back. So, Todd, are you able to connect now?

Bourcier: I think so. Can you hear me?

Hayes: We can hear you, Todd.

Bourcier: Wonderful. Regarding the original question, I guess is, they're asking this set of 48 that we have, what's the overlap with the old data set that industry came up with? There's overlap. I can't give an exact number but there is overlap. There's some drug classes we've seen in the 48 that exist in the older data set. But there's also brand-new targets. There's also brand-new targets in this 48, but I want to emphasize a point here I think is rather important. The elements within that weight of evidence are independent of what the target is you're going after or the drug class. We're looking at basic mechanisms across the genesis which are true regardless of what the initiating stimulus is. So, I've heard this argument before, what about new drugs that are going to come in the future? How representative are the drugs today of the drugs in the future? Well, the target is going to be different, but the concept will be the same. If we focus on what are the concepts we need to look at in a weight of evidence the evaluation, shouldn't be afraid of what the next new target is going to be. So, I do want to make that particular point.

Hayes: Okay. I've got another question for Frank. [Indiscernible] how might it be applied to molecules with new modalities while still identifying the mechanism where the cancer bioassay may still be needed?

Sistare: I'm actually struggling to completely understand. By new modalities, so we're not talking about small molecules? Are we talking about cell therapy and things like that? We have F6 which kind of speaks to the whole biotechnology guidance. S1 is really focused on chemicals. S6 is already sort of inviting a white paper-based approach dialogue with agency, we understand this is a unique modality. We've got to think creatively, how do we address tumorigenic risk? That's really out of the scope of s1 which is chemically oriented. I hope I'm answering that question.

Hayes: What I'm going to do, Frank, is ask the questioner to clarify the question so that we may be able to get a better answer. So, I'm going to move on to the next question, and it says I'm having some trouble with how this approach could work for botanicals. I think, Sam, that probably hits you pretty good.

Cohen: The FEMA expert panel deals with natural flavor complexes which are derived from botanicals. There was a publication in 2018 as to how that can be approached. You do it by component parts. You look at what the composition of the botanical is and evaluate it. If you need toxicity data, the approach would be if you do the entire botanical or the extract, you look at short term assays as a predictor of what's going on for the long term.

Hayes: They go on to say a company could do a 90-day study with a very clean preparation and find no evidence of cell proliferation but the marketed product from them or competitor may be somewhat dirtier that could cause proliferation. If bioassays were not conducted on botanicals, potentially carcinogenicity of dirtier versions of the same botanical might be missed. Can the paradigm be changed to address these types of substances?

Cohen: If it's in food, as a flavoring agent in things, there's specifications so you can't have a, quote, dirty substance put in. When you come to dietary supplements for which there's no regulation and somebody from FDA might discuss this more, there's no definition of what the composition is and there's no definition of what toxicity is with these agents at all until there's actual evidence in the human population of human safety problems. So, the botanicals that are used to supplement are essentially not regulated.

Hayes: Any comments from our FDA panelist?

Zang: This is Janet. I want to echo what Sam just mentioned for food additives or food ingredients. We typically require chemical characterizations and also require at least three different preparations with chemical profile sent to us.

Hayes: Okay. Thank you. Next question is how do we address false positives in general? In labeling a product as a carcinogen without conducting a bioassay.

Cohen: As I was trying to point out, this is the big issue that's got to be addressed if it we're going to deal not only with the current two-year bioassay or short term or other kinds of assays. If you're going to label something as a possible carcinogen just because it's positive in a short-term screen even though it may not be relevant to humans by mode of action or exposure levels, then this whole approach is going to be a disaster. Essentially the false positives are false positives because they're not relevant to human exposures.

Hayes: Any other comment?

Sistare: The only thing I'd add, to call it a false positive you'd have to know the truth. So, I think you get to truth when you understand mechanism. Any signal really needs to be understood sufficiently at a mechanistic level to be able to put it in a false positive or a true positive category. What it comes down to is just good, old-fashioned investigative toxicology.

Boobis: It does come down to formulization as well. The question is what is the question we're trying to address? If this is an environmental contaminant, your bar will be different. If it's a pesticide. So, I think it goes back to what Doug was saying. It really is about problem formulation at the front end before we then think about what's the degree of latitude in getting a false positive that we can live with? It would be different.

Hayes: Any other comments or thoughts? Another question or comment, there are toxic chemicals that are not carcinogenic. How do they fit into your model of toxicity-based mechanisms?

Cohen: There's a lot of toxicities that occur that are unrelated to cytotoxicity and regenerative proliferation. Such as [indiscernible] have nothing to do with cell proliferation. And they fit in very well. You focus on the toxicity issue, it's not a cancer issue.

Hayes: Next question. Without demonstrating cause and effect in the two-year bioassay, how do you address the effects of exposure duration? Chronic, low dose exposure, and timing. Parental, peritoneal exposure.

Cohen: Wally, can you clarify that? I'm not sure what that question is asking?

Bourcier: It's prenatal and perinatal exposure. It's the duration--

Hayes: It's probably more of a reproductive, when does exposure occur and how long does it occur?

Wolf: So again, it's in the context of the problem formulation, what's a risk decision you're trying to understand? As far as risk assessments for chronic low dose exposure, that's basically what we do in risk assessment, extrapolate from the exposure situations in the models we use to what the human exposures are. And so, for environmental contaminants and crop protection chemicals and things like that that the exposure to humans is low, we can model that through different models such as what Professor Boobis showed at the benchmark dose modeling and that so we can extrapolate to lower dose exposures. I think there's challenges. I think with the *in utero* and perinatal exposure, we do accommodate some of that within the context of our testing paradigms. Those are different kinds of studies and I'd suggest that that might not be within the scope of this particular discussion. Because in this particular discussion, we're really focused on the traditional two-year rodent to 18-month, two-year rodent bioassay. Understanding what happens *in utero* and during development, that's a different type of evaluation. And relationship to biological processes. But a lot of the conceptual approaches we talked about, understanding exposure to the fetus, to the dam, to the mother, and the kinetics, how it gets to, through the placenta or the milk, that's still the same and just as critical.

Hayes: Okay. Next question. In carcinogenicity evaluations, should an extra uncertainty factor be applied if a point of departure is derived from a 13-week study instead of from a two-year bioassay?

Cohen: No.

Hayes: Got one no. Do I have more nos?

Wolf: So, can I expand on that? So, the additional, if you look, at least for the EPA and policy-based uncertainty factors, interspecies, LOEL to NOEL extrapolation, I think what's talked about is a database uncertainty factor. But the issue here would be, I agree with Sam, that there be no need for that additional uncertainty factor because you are making the decision that that study is not necessary for the database and so by using the weight of evidence approaches Professor Boobis looked at, the historical context that Dr. Sistare looked at, and all the data that's available, you're saying we don't need this additional study. So, that additional uncertainty factor that is part of policy would not be required.

Hayes: Any other comments or thoughts on the extra additional uncertainty factor?

Zang: I just wanted to add one point. In regarding to this question, we also wanted to make sure that we understand the pharmacokinetics, toxicokinetics. That information will be very helpful to address the uncertainties.

Hayes: Thank you.

Bourcier: From the pharmaceutical point of view, if we do adopt this particular approach, similar to what we do at biologics, when we do have this weight of evidence document that essentially a carcinogenic risk from this chemical, if the regulator and industry agree or concludes that we know enough about carcinogenic risk from this molecule, we do not need further information, then yes, we made that decision and there's no need for an additional factor to be introduced into any margin calculation of such. That's only in cases where the regulator concludes, yes, we know enough now even without a two-year study, for example, to make the call on what the carcinogenic risk is. But it all depends on that weight of evidence evaluation and how persuasive the evidence is.

Hayes: Next question. How do you assess the role of multiple exposures to humans of different substances at low doses within the single high dose model that are used for risk assessment? Al is going to comment on that.

Boobis: I think the approach that's being proposed is pretty well the only one that will help address that, which is the idea we've got a cumulative effect of multiple chemicals acting by either the same or related mechanisms, so I think what we've got to do is look at the mode of action for the different chemicals and think about how they might add up to create precursory effects. So, for example, if you've got nuclear receptor activation as being a target of concern, do we have several of the chemicals to which we're coexposed which all activate the same receptor, and we can then factor that in to our assessment. I think trying to do it by a bioassay is doomed to failure because that's where we've been singularly unsuccessful to date in addressing the potential risk of multiple exposures. We can only do it by moving back toward the key events and a mechanistic evidence-based approach.

Hayes: Any other comments or thoughts from the panel?

Cohen: I agree with Alan entirely. The only way you can do it is going to be a mode of action analysis and there might be interactions not only with chemicals that act on the same receptor but different receptors that are modified. For example, a chemical that acts on a car receptor is going to modify the metabolism of other chemicals that don't act on the car receptor. I think one of the things that people need to be reminded, short term assays, one can evaluate complex mixtures, like you can for natural flavor complexes. There's a couple old studies that looked at initiation promotion in [indiscernible] laboratory in Japan I think back in the '80s that looked at two sets of experiments. One looked at 25 chemicals and the other one was 50 chemicals that were administered at their ADI level together. And they found absolutely no effect.

Hayes: This brings us back to botanicals. It says the NTP has done several bioassays on botanicals. Some of those have shown positive results. Aloe, for example, results of 90-day studies with clean aloe preparations have been negative. I think it would benefit the dietary supplement industry if the paradigm could accommodate botanicals.

Cohen: Aloe, one in specifically I'd like to address is the NTP did their bioassay on an extract of aloe that would not be acceptable for commercial purposes, either as a food ingredient or as a cosmetic because they did not eliminate the anthraquinones, which are well-known genotoxic carcinogens and are present in aloe by itself at very high levels, and it clearly explained why aloe was carcinogenic in that bioassay. If you meet specifications, the anthraquinone levels of cosmetics and food ingredients have to be less than 1 part per million of anthraquinones. These are going to have no activity whatsoever.

Hayes: Thank you, Sam. And that is the end of the questions that I have. We'll give people another 30 seconds or so if there are any more questions. Otherwise let me, something just came in. To follow up on the POD from the 13-week study, the waiver I understand from today's focus, focusing on carcinogenic potential, from qualitative perspective I think uncertainty factors should be considered carefully as a database across more than 100 agrochemicals indicating uncertainty factors for short term to long term studies may be needed. Again, this was a comment.

Boobis: In Switzerland, published several papers now looking at specifically the pesticide database of approved pesticides in Europe shows somewhat more counterintuitively that there's no change in the points of departure from a 90-day study to a two-year study for toxicity. This is not cancer. This is points of departure for the lowest effect. Once you correct part of the consumption intake of the animals. So that I think we should think about a possible extrapolation factor from 90-day to two year. I think we'd have to take into account that would be only necessary if an overall evaluation of toxicity suggested it was required. I don't think it's a particular issue for carcinogens.

Hayes: Anybody else, comment?

Sistare: I don't have a comment, but I have a question I'd like to pose to Doug if I have time.

Hayes: Go ahead.

Sistare: Doug, if I caught one of your slides correctly, that you had showed where you have a list of waiver requests and then waiver grants for carcinogen studies. Do you have any insight into those waivers that are requested and not granted and then ultimately the outcome of those studies? Is there any analysis that could be done? You probably can't do it from your vantage point but I'm wondering if on the other side, has any analysis like that been done?

Wolf: Not to my knowledge. I saw Greg Ackerman was online, so I don't know if he'd be able to comment on that. There's a number of projects going on. One of them is to do a retrospective on the accepted waivers. It's one of those really significant activities that the EPA has conducted and it's a really good news story as you can see from that one slide. But I don't have the specifics on the rationale for rejections other than the one that they rejected. They're usually very clear as to what their rationale is. That probably could be done and find out what the common rationale is. For the waivers I've seen rejected, usually around a lack of sufficient information on the margin of exposure for the particular use but I don't know for those chronic studies. I don't know if Greg was going to be able to weigh in or not. Or anybody from EPA that's been involved.

Hayes: Thanks. Sam, question for you. Please comment on how thresholds, NOELs for cell proliferation, immunosuppression, hormonal perturbation compare to those for genotoxic carcinogens. What generalization, if any, can be made?

Cohen: The argument about DNA reactive carcinogens is still an ongoing one, whether there is a threshold for those. Generally, for those chemicals, you don't apply a NOEL or BDL approach. Because it's assumed that it's going to go down to low doses. In contrast to chemicals that are cell proliferation, cytotoxicity, hormonal changes or immunosuppression, so the approach is totally different which is why I focus my talk on non-DNA reactive carcinogens. That battle is still to be fought.

Hayes: And I think Gary Williams and some folks like that are trying to sort through that.

Bourcier: I have a question for Sam. This is Todd Bourcier, FDA. Sam, as you know, in developing pharmaceuticals, the toxicology studies that are done, they assess proliferative markers by looking at the slides. How important do you think it is to have established a labeling index or other more sensitive means of picking up proliferation organ by organ in at least one of those studies?

Cohen: I think it really is dependent on the organ. For the liver, data shown by Bob [indiscernible] and the others at NTP is a screening method for basic histology and organ weight was adequate. And actually, for kidney, it probably is also, although there are more sensitive ones, there's a couple cases where that's true. For other tissues like lung and urinary bladder, and a couple instances kidney, you really need the labeling index to pick up changes that are more subtle than just the histology. So, the histology is going to be relying on hyperplasia which may appear later, whereas you can pick up the labeling index much earlier. There are a few studies, 13-week studies where there's no evidence of hyperplasia at all and yet the labeling index has been high. That's particularly important for an assessment at different time points. We're all familiar with the liver where there's a lot of increased labeling index at one week but then it goes away at two weeks or four weeks and yet the livers are enlarged so you've got more cells as Doug pointed out earlier. But the trickier one is the lung. The lung frequently doesn't show any evidence of hyperplasia through 13 weeks. In fact, sometimes even through six months. Yet if you do a labeling index at one week, not 13 weeks but one week, you see that increase in labeling index very distinctly. That's been true for a number of classes of chemicals, industrial, agrochemical, food substances, even pharmaceuticals. I think the labeling index would be a useful addition and it doesn't require anything more. You don't have to do a BRDU injection to do this. You can use the same blocks from the animals and tissues. It's pretty straightforward, but you have to standardize the methodology for each organ.

Bourcier: Thank you, Sam. This might be a naive question, but when you see an increased proliferative index in a given organ, is it fair to say that's a reasonable reflection of stem cell proliferation in those organs or is it somatic proliferation?

Cohen: Usually it reflects stem cell or pluripotential cell but there are some exceptions. Lead nitrate is the one that comes to mind.

Bourcier: So, there's probably little value in distinguishing between those proliferation occurring in those two cell types?

Cohen: I think for practical purposes, it probably isn't necessary.

Hayes: Okay. I'm going to close down the roundtable discussion and thank Doug and Sam and Frank and Alan for just outstanding presentations that really brought us to the point where

I think we all need to seriously think about the need for, the purpose of, and where the cancer bioassay is headed in the 21st century. And also, to thank Janet and the Todd for taking part in the panel. We have a series of a couple additional slides that if we could get brought up, Suzie will close out the session.

Fitzpatrick: Thanks again to everyone. I think what FDA was trying to do is to start a dialogue on how we can update this valuable regulatory tool to give us answers we need in the 21st century. We're hoping this is just the beginning of a discussion between FDA and our stakeholders on how we can do this and that will be continued in other venues and work groups. The colloquium also has some exciting future workshops that we hope you'll all be interested in. In April, we're going to talk about Bioprinting as a Tool for Testing. In May, we're going to talk about *In Silico* and *In Vitro* Computational Modeling and Methods for Food and Cosmetic Safety. On behalf of the FDA, thank you to the speakers. Thank you to Wally. A special thanks to our AV people, Ben and Donna, for doing this in the midst of a snowstorm. And of course, Betty, whose steady hand and patience and hard work made this all possible. Thanks again. The slides will be available, as you can see, on the SOT website. Thanks to SOT for being a great partner as always in transforming regulatory tools for the 21st century. And thank you all. Keep in touch for our next symposium. Thanks.