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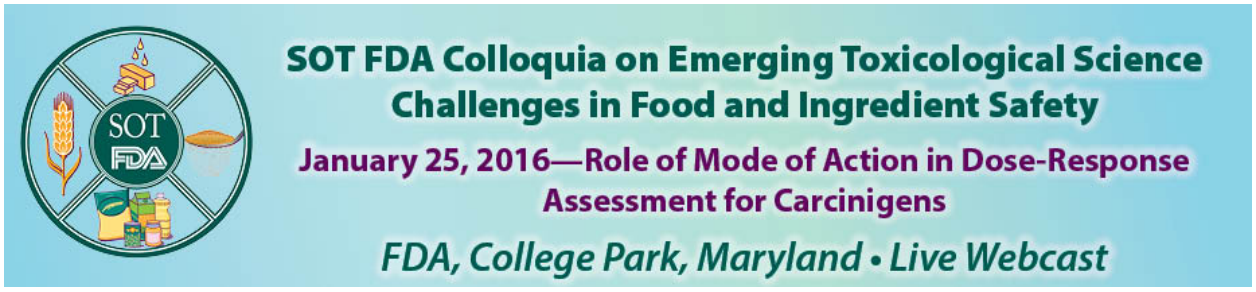

Role of Mode of Action in Dose-Response Assessment for Carcinogens

Conducted by webcast only due to a snowstorm.

Schedule

8:30 AM–8:40 AM	**Welcome, Overview, and Introductions** Suzanne Fitzpatrick, US FDA/CFSAN, College Park, MD
8:40 AM–9:20 AM	**The Role of Mode of Action in Dose-Response Assessments: Recommendations from the 2009 NRC Report "Science and Decisions."** Lauren Zeise, NRC Committee Member, Berkeley, CA
9:20 AM–10:10 AM	**Mode of Action, Distinguishing between Mode and Mechanism of Action, and Some Key Events for MOA** Michael Dourson, TERA, Cincinnati, OH
10:10 AM–10:25 AM	**Break**
10:25 AM–11:05 AM	**The Mutagenic Mode of Action and the Choices for the Dose-Response Analysis** Rita Schoeny, US EPA (retired), Washington, DC
11:05 AM–11:45 AM	**Risk21 Quantitative Key Events Dose-Response Framework** J. Craig Rowlands, Dow Chemical Company, Midlands, MI
11:45 AM–12:50 PM	**Roundtable Discussion** Elaine Faustman, University of Washington, Seattle, WA, Moderator All speakers

8:30 am–8:35 am Welcome, Overview, and Introductions—Betty Eidemiller, SOT HQ, Reston, VA

Welcome, everyone, we appreciate your participation today. Today we are featuring the role of mode of action in dose response assessment for carcinogens. This is chaired by Suzanne Fitzpatrick, and we seem to be having some technical difficulties getting her on. I'm Betty Eidemiller from SOT Headquarters and again we want to welcome everyone. These colloquia are a joint product of CFSAN, FDA and the Society of Toxicology. Suzy was going to point out something about this Twitter from Dr. Susan Mayne. SOT's mission is to create a healthier and safer world by advancing the science and increasing the impact of toxicology and is very pleased to work with FDA to produce this series that will focus on a very important topic and give us an opportunity to share in-depth knowledge. These sessions are designed for FDA employees. And the public is also able to access the materials during the webcast and as recordings. We have had fantastic participation and once again we thank you for participating today.

The following colloquium will be March 29 and will feature State of the Art in the Cramer Classification Scheme and Threshold of Toxicological Concern. That will be followed in late April or early May by a colloquium featuring the Safety Assessment Approaches to Sensitive Subpopulations. The organizing committee that put all of these series together is on this slide. We especially thank Ivan Rusyn who chairs the group. This is a combination of staff from FDA and SOT, as you can see on the list. And Elaine Faustman is going to moderate and I will turn this over to her.

8:35 am–8:40 am Introduction for Dr. Lauren Zeise—Elaine Faustman, University of Washington, Seattle, WA

Thank you, Betty. And to Ivan, and extended thanks to Suzy Fitzpatrick for putting this opportunity to gather. I would like to introduce our first speaker. Dr. Lauren Zeise. She will go over the dose response assessment. She will also go over the 2009 NRC report "Science and Decisions". She is the acting director and she has had a long association with the office in California since its inception 1991. She spent three years as deputy director and she has spent 21 years in reproductive and cancer hazards assessment. We are very excited for her role in the National Academy of Sciences committee, seeing where we are at and how we responded to this challenge. Please welcome Dr. Lauren Zeise.

8:40 am–9:20 am The Role of Mode of Action in Dose-Response Assessments: Recommendations from the 2009 NRC Report "Science and Decisions"— Lauren Zeise, NRC Committee Member, Berkeley, CA

Thank you and good morning everyone. I was asked to give an overview of 19 of the 2009 Science and Decision reports, particularly regarding mode of action and dose-response assessments. I am giving this presentation as an NRC committee member.

It is 5:41 in the morning in California, so I'm speaking not during my employment or reflecting reviews of Cal EPA. But I really want to reflect as a committee member, the approach that the committee has towards dose-response analysis and considering mode of action.

For the outline, we will talk about key considerations by the committee, and the committee framework and finally recent developments on constructing dose-response relationships and the thoughts of the committee in this regard.

Here is a backdrop for the committee's consideration of dose response. These are the observations with respect how dose response is connected. And how it is done for non-cancer and cancer risk assessment. On the non-cancer side, there are no risk measures are produced. For example, there are hazard indices, reference doses, and margins of exposure that look at the measures of toxicological activity. And there is a ratio between that measure of activity and environmental exposure for the hazard indices, and you would look at a reference does for which it was unanticipated to have an insignificant dose at that level and compare it to the level of exposure in the environment. And look at the ratio of those two indices.
Here we have linearity. And finally, uncertainties that are not distinguished from other adjustments. This is the overall uncertainty factor.

On the cancer side you can see individual variability and human cancer risk is not addressed and the analysis is based on animal data. There are low-dose nonlinear carcinogens -- this is the hazard index approach for non-cancer. There is not an explicit characterization for uncertainty of the dose-response from the animal data.

The NRC looked at key consideration in dose responses. They looked at the relationships that you have a chemical stressor that produces a dose response in an individual. And this converts to a dose response relationship to a population. And one of the drivers, is the background exposure. The chemicals that affect the process, stressors that produce toxicity as well as endogenous and exogenous exposures -- all of the exposures in the environment. Then you also have the health and disease status of the individual things like their genetics, age so forth. And this is the dose response relationship how it will differ across the individual and that backdrop is mode of action and how is the chemical operating?

First, we will start to think about the background. This figure depicts a varying background in an individual. Some people have higher exposure and of course they are more vulnerable to chemicals. And this may tip them over into response.

And here is one example of background attendant dose-response. This is seen in the animal studies. The black bar represents the response to Bisphenol A when the animal receives a diet with soy. This is where they are receiving normal diet. And you can see the dose response in this case.

And if we look and consider the background in cancer -- here you have people where have very different cancer train town --, and lifestyle. And you can see, the magnitude different -- difference associated with this cancer. If we consider migrant studies as you can see -- it is different background incidence. This is the host country versus what they eventually develop once they are experiencing the same lifestyles and other factors in the country in which they migrated. There are very different -- this is compared to what you have in a background incident in Japan. And for breast cancer you can see a great increase once they migrate and a diet -- adopt the American lifestyle. But you can see a drop-in stomach cancer. Here are contributions from the environment that resulted in these changes. And how chemical exposure might be is associated with prostate cancer. I apologize but I just lost all of my slides. I apologize. Please give me one moment.

Background exposure provides the way of thinking and interpretation of data from Tox21. This is the approach to toxicity where you have cell assays. This is for looking at dose response for individual chemicals and combining these with whole animal studies. It is population-based with exposure data.

Here is another example. This is coming from the report from the national research Council, there were several examples in that report. Where chemicals at below-threshold levels were combined with other chemicals. This disturbed androgen action and reproductive outcome below-threshold doses. Where you would not see this affect the animals. But this was combined in a mixture you would see an effect.

This just lays out all of the details on what was seen. Where individual compounds and a mixture resulted in interference with androgen-mediated development and toxicological outcomes. And this gives you the overall simple way of thinking about what is happening with the dose response. Here we had a threshold relationship -- but then with a background exposure you have the Y axis translated. You have a case where there is perhaps a dose-response relationship that had a linear low dose component without the present of background threshold. These are individual -- individuals that moved into a range of response. Now we have variability. And mode of action. There are a lot of different factors. On how a -- an individual will respond. Here we have gauged -- age and gender and so forth. There is variability among the factors across the population when you think about going from an animal study to a heterogeneous population that variability drives the shape of the dose response. And for the homogeneous animal that are tested, for most studies they differ very little. To the extent that the individual animal in the study differ from each other.

And consideration of variability in context of the National Academy of Science 2007 Toxicity Testing in 21st century report we often see this diagram. This is what affects the ability to respond to a -- it influences the challenge. In the toxicological pathway. And this could be the adaptive response in a population. But there is a caveat to this diagram that acknowledges that variability and the potential – response to these perturbations. And it is often missed in some of the discussions. And you can see data from toxicity tests.

This is where the host is unable to adapt to the change. You can have toxicity and disease. And this is just a reminder when you consider the interpretation of these results -- on the human status in terms of these different factors associated with these different variabilities should be taken into consideration. And there are many new tools to interrogate variability. There is some *in vitro* methods. You can see that sometime in March SOT will it explore this further. Different ways in which susceptibility can be described. I just wanted to highlight there are various methods that can be used to explore inter-individual variability's. Here you have heterogeneous animals. And various ways that you can approach it in *in vitro*.

The implication for dose response. This is show weighing different subpopulations. Which have varying susceptibility and environmental exposures. And the implication for dose response is once again, instead of a sharp dose response that one might see. Here is a set of individuals as you look across the subpopulation you have this relationship. The 2009 framework took into account this background with individual variability and mode of action. And you can see in this diagram, the figure from the beginning. Where the risk is in determine by the individual's biological makeup and health status. Here is the background exposure -- and the difference among the individual will read -- shaped the dose response. So how do we depict these risks? We take into account, mode of action. And the background processes. To understand the implication of background exposure and variability on dose response and developing population dose response. On the left-hand corner it shows a median estimate curve per dose response.

We also can see the individual risk. And we can focus on describing the effects in sensitive populations and consider that in the context of what you see for the individual. And developing

uncertainty relationship. This example is uncertainty in dose associated with a sensitive individual. And what their dose might be. And realizing there is a great deal of uncertainty. And here is another example of the conceptual model. The first cases where you have an individual that has a threshold. And that background exposure and see if it is high. It can result in a linear dose response. The second case is that typical case. When one assumes that the threshold is at the population level. You have a background dose that is considerably below what the dose that is associated with the individual responses. And that the individual response -- dose responses linear at the population dose-response level.

And the recommendation -- this was a formal systematic assessment of the human status. Looking at the background and exposures in the population and using mode of action. And to identify the modeling approach to dose-response relationships. And also develop probabilistic risk for the threshold. Nonlinear. This is where you define the RFD as a risk-specific dose for the population. And you develop uncertainties and characterization for that into the cancer dose-response modeling.

There are various suggestions around implementation. Which we will go into more detail in a minute. And here are a few comments about constructing the dose response mode of action. Here you have cancer and non-cancer. They are not distinguished as separate but there is a unified approach to consider the assessment beginning with assembling the data. And having endpoints -- that are evaluated for each sensitive endpoint. And to assess what could be driving the dose response relationship. And it is evaluated in that mode of action. And including consideration of endogenous and exogenous -- using that underlying consideration to assist vulnerable population. And then to use that to select a model for approaching the dose. And using the mode of action to guide the modeling.

Here are some examples. Here are a few diagnostic questions. And this is what you should consider for the mode of action. Here you can see it underlines they individual endpoint. And this is considering background incidence. And considering whether or not there are characterized in the human population precursor effects. And look at environmental, chemicals -- both that could interact. And that could provide the basis for additional background information. There are various conceptual examples. Here is a conceptual model. And this is that committee -- and this is what the committee laid out in the report. I also want to mention, for that heterogeneous individual threshold -- here you can see a good example. They also laid out asthma-exacerbating chemicals. This is basically a bottom-up approach.

We also laid out in the report, an example on how you would calculate low doses or nonlinear RFDs. The IPCS – Dr. Chiu at the summer FDA symposium, they gave us a very nice overview approach. Coming out for a dose response relationship. And there is also software that has been developed.

For low dose -- I apologize but I lost my slides again.

This is a quantitative risk assessment that are derived -- how to distinguish between risk and vulnerability.

Here it is laid out for human variability. This is uncertainty and articulation and extrapolations.

And finally, in the report -- here is an example where you have a linear dose response relationship at both the human and population level.

So concluding, mode of action data are critical. In the NRC's 2009 vision on dose-response relationships. And the treatment of cancer and non-cancer evaluations. And here are some examples. But more case examples are clearly needed at the chemical-specific level. To account for background processes in human variability in model selection. This is going to be very useful to provide guidance. And finally, through the IPCS, there has been a great deal of process for developing the assessment. Thank you so much. I look forward to taking your questions.

Faustman: Thank you so much. It is my understanding that the questions are going to be handled at the end. Will somebody clarify that for me? Because we have so many people on the phone.

Eidemiller: I think that may be the case. But right now we do not have any questions in the chat the church -- chat feature right now.

David: I do not think we have any questions.

Faustman: But we do encourage people to start thinking about their questions. Because one challenge for a webinar, is to make sure that we get your questions. And that we also know who the question is directed to especially if it is for a specific speaker. This will help us sort through your questions. Because we do have over 500 people who have registered for this webinar. And thank you, for your presentation.

I think you will also hear a couple of case studies.

It is my pleasure to introduce, Dr. Michael Dourson. He is dedicated to the best use of the risk assessment. This recently came to the University of Cincinnati. And we are excited about this do opportunity. Before founding TERA, in 1995, he also had a leadership role in dose and he is a charter member of the risk assessment forum, and chief of the group that helped to create the IRIS integrative system. Thank you for your presentation, to distinguish between mode and action.

9:20 am–10:10 am Mode of Action, Distinguishing between Mode and Mechanism of Action, and Some Key Events for MOA—Michael Dourson, TERA, Cincinnati, OH

Top of the morning to all of you, gentle people. My name is Mike, I am from the University of Cincinnati. I want to thank you for putting this all together. It is a challenge to listen to everybody talk and not being able to contribute. I would like to talk about this launch point. There are groups that are working along in the line of science in decisions. Including the government and nonprofit industry. We have 40 cases right now. So, there is a lot of activity going on. And they do fall into as Moran said, mode of action. I do not have access to the webinar, so I am going to go through my slides. Please go to slide number three.

I want to talk about research that has been funded by different groups. And the mission, as a nonprofit organization, we are supporting the protection of public health. We are also working with our colleagues at FDA. Please go to the next slide. This is out of USDA. This is the mechanism of action, for toxicity. What the EPA and others have done…there is a framework that was developed internationally. This is to look at, can we distinguish key events within this mechanism of toxicity? And the answer almost always has to be yes. And the reason that is the case, because if you do not have any of this information what do you do? Go back to 1954.

Here is the linear dose response that came out of [Indiscernible]. This is what we do as the default. And so, if we have any kind of data whether it is just one key event it becomes the default. And if we have some information it is better than the default. Here is the black box of [Indiscernible]. Many years ago, we had a situation, of exposure to [Indiscernible]. There was little data. And we had exposure and we had the presumed outcome. What ensued was $10 million worth of work, to try to get inside of this black box to figure out what is going on. I think collectively everyone was successful. There is additional work on Pharma Co.

But the point is that we got inside the black box. And we did this through, the idea on how does the chemical effect the body? What is the early effect? And what is the critical in effect? And we had the scientific community -- please go to slide number six. When you look at this you might say, it does make sense. Here we have normal biological functions. Here is biological [Indiscernible] that protects themselves with the extra margins. Why have the extra margins? We do not want to have to look at their genes all of the time. So here is a normal biological function. This is what the body can normally take care of. Here is the SLT about four years ago. Where we talked about regulation and various levels of formaldehyde. Here are additional protective enzymes. This is what the cell was regulating. And this is the cell how it was [Indiscernible]. And it will regulate it as needed. Here is the black box. You have this normal biological function and you have these early cell adaptations. This is [Indiscernible] and you can measure adaptation. However, there is a point where you have to go beyond that. And it can be different for different people. You have variability is just like what Lauren talked about. Here is the outcome pathway. If you look at the next slide, which is looking at NAS. It starts with the chemical then it goes into how did you measure pharmacokinetics? You can look at the molecular target. And some of these are more targets for exposure. You can get leakages to hemoglobin. And this is a good marker of exposure. We have [Indiscernible] that has the adverse effect. And this is certainly a marker. What is the birth defect? So, we are having some early thinking along these lines. This slide shows the model. This is similar to what we have been thinking. But it does lay this out nicely. Please go to the next slide. There has been this change in acronyms. I apologize but I have been part of this tradition. We thought that ADI was old so now we call it the reference dose. I know the older people at FDA are rolling their eyes and saying it is the same thing. But the point is that we change our terminology. Sometimes it is not appropriate. But sometimes it does make sense going to the reference donors. Then you can build data in. And this is what people have done. All of the sudden we are removing uncertainty factors and why? Because we have data and that is a good thing. My first thought was, it is the same thing, but it really is not. This is the inherent structure that we are dealing with. It is chemical agnostics. However, the mode of action reflects how the chemical skips or plays the body or however you want to say it. This is what the chemical is doing to the body. And the underlining SOP. And it is very much chemical specific and there is a difference. From a risk perspective, it is akin to the difference between kinetics and dynamics. Kinetics is what the body does with the chemical. And the dynamics is what the chemical does to the body.

Please go to the next slide. Here is the threshold. One thing that is clear to me, you cannot determine the threshold without modeling. And Kenny Krupp can show you the threshold and vice versa. We have to understand the biology and the math is just the tool. If you look at this data, it looks like a straight line. So now what? How do you get underneath that? We have to try and understand the biology. And of course, understanding the biology is the idea of understanding what is the structure of the body. What are the pathways for that specific species whether they are human? And we have to understand this idea of threshold. And of course, if you really want to get into it you have to understand the mechanism of the action. And I will show you some examples. We also have this idea of, presumption of dose response curve. This is one area where we need to expand. There is the assumption for the linear graph. There is a

chance versus variability in the population. And it is due to this chance. So, it is probably not true that these are exclusive with one another. Because they are different. And if we want to quantify one versus the other and the problem is [Indiscernible]. Go to your typical dose response approach in this is slide number 10. We have all seen these 100 times. You have all of this nice data. And theoretical data always looks good. You also have your benchmark dose. But I think we understand this benchmark dose. We can pick a benchmark response or whatever will fit the data at this point. Then we can do extrapolation. And EPA is taking the ruler to the benchmark, but you do not have to do that. But the Canadians will take that point of action they go with a larger margin of exposure. But that is another way to do this. But that is not the only way you can look at it. And now look at this. You can see all sorts of things. Here you have the dose response curve. And here is the hermetic curve and this is different than nutrition. And if you do not get the chemical in this case you do not die. If you do not drink the water. And that is why these curves are different. But there is plenty of data for this, but it is just the matter of trying to understand it. And we do have adaptation. And you have the normal dose response toxicity curve. Then you get this [Indiscernible]. We have another one? And the only way to understand this, is to go to the mode of action or mechanism. To understand this. And if you are a risk assessment evaluator for a complex study and each study has 2000 pages. With all the individual animal data. You will see this curve all of the time. This is called toxicity masking. It gets masked by a more severe effect. Then you can see these are two different dose response.

These are several effects that are coming together. And the next slide is number 12. This is the framework. Here is the mode of action. Identify key events. Look for experimental support. And you have people that will put together and this is something that we are routinely doing. Here you have your mode of action. And it really does not look good. And you cannot make that presumption. And so therefore, we do not have to worry about putting these tables together. But I think you have to do that because this is the new expectation. Next thing is the modified [Indiscernible] are important too.

Martha More came out with the outside creative. She did a lot of good work and try to understand the mutation and dose response related to the tumor response. This is actually getting the data and it was quite helpful. And they do have new publications that can be helpful. Here is the future Tox 21 and the vision statement does have predictive analysis. This is where I say, it is not predictive. We cannot predict these solvents. We should become more efficient and more informative. And we need to understand mode of action. And improve the scientific risk assessment. And in order to do that there are a number of things that need to be worked out. Please go to the next slide. This is the idea of system biology. This is the idea of general hypothesis. If we give the chemical at 10:00 PM to the rat we may see something that is consistent with the biology. And that is great. Now we can put together a hypothesis on how this cell is changing. And we can start to test the hypothesis. We start with the endpoint identification. We can start to look at mixtures in a very systematic way. And of course, the interpretation might be difficult. But *in vivo* we have a lot of good work out of Simmons lab where they did the *in vivo* test of mixtures. With about four or five different solvents. I do not know if we had this mixture in the environment are not. But there is certainly a lot of different mixtures that cannot be tested because of time and resource. Look at biomarkers. This is what we used to measure exposures. This is where we have some exciting work to do if we can help characterize these adverse outcome pathways. When a chemical comes in we can get a better handle on it. I do not want to say without a lot of resources but in a way that is systematic and quicker. What I want to do is give you three examples. And I want to try to get us back on time. I apologize but I cannot get on the WebEx. Do I have 10 more minutes?

This is Elaine, I think you have 10 more minutes.

Thank you. I will quickly go through some of these examples. When you think about this mode of action, they are not easy to sort through. So, when you look at [Indiscernible] there is a lot of evidence. There is evidence on [Indiscernible] stress and other kinds of things. One thing that you can do is look at mutation in relationship to tumors. You can do that comparison, but it is not clear-cut. So that does not mean you fail if you were not able to measure it. But the point is that you can look at different types of key events. And as you do this you do not necessarily get easy answers nor agreements. And that is why this is difficult. Look at this publication. This is our FDA colleagues that are trying to get to this idea that do we have hormone changes that causes [Indiscernible] in the thyroid. And their conclusion was that the evidence did not push it that way. But if you look at the regulation, out of their own paper it did not look that way at all these to the risk assessment people. Here you have a hormone protein associated with [Indiscernible]. You can have these data sets and you were going to have a lot of these data sets and people will have to get together and talk them through. You can even have just one group doing it. In the case of mode of action for [Indiscernible] look at this slide it is a dual action. What you have is two mode of actions. Here is a 19 data point and we do have the extended data set right now. But the point is that you can model these data. And you can have a mode of action and it did not work. And with 19 points we could not get it to work. But we were able to use EPA's method to give us a good look. And those are the results right there.

And the same is true with tumors. FDA colleagues came up with a great study. But there was missing information. We were able to gather additional information from our Japanese colleagues. And we are stepping through this mode of action right now. And the point is the science changes and the mode of action information accumulates. And as it does this we can keep looking at this data. And I go again to this idea of working collaboratively. Because this is too much for one person to come up with this a complete picture. This is the mode of action. And they laid it all out. We have been able to put more data into this and start to make judgments as to which pathways should be precluded and this is just another example.

Here we have metabolic saturation. Here is EPA slide. Please go to the mode of action for trans-fat, the interesting thing right here. I thought we had complexity with [Indiscernible], but this is even more complex. Because of the macro nutrient. And to judge it, you need to change the nutrient. If you do not you have changed the diet. Here is LDL. And if you go to the next slide, what we have right here, there are three kinds of data. And the CLA and the dose response curve is confusing. When you look at this you are probably going to say, what is going on? And the question is best doing the math of it? No, it is the biology. Here you have a low density [Indiscernible]. And you have this decrease clearance -- it is in the green box to the left. This is where the biology was focused. But if you look at the biology and additional data, you can get different kinds of modeling. In this case we had [Indiscernible] models. And this is the overall pattern and if you look at the next slide -- I think this is slide 55. This is the low dose model. Then you get a low dose model with [Indiscernible] bars. You have to understand the low dose model first and there is a summary of TSA and I will let you look at that separately. I think we are back on time.

Mode of action is important. It is better than default. Anybody who works with risk assessment probably will not argued with that. Here are the key steps. They have to line up. If you are going to say chemical causes DNA [Indiscernible] and mutation or tumors, that is a good mode of action lineup. Now what you have to do is look to see if the adducts lineup before the mutation. Because we can get mutations at a high dose. However, the mode of action, you do not have to go to the mechanism but if you have it that is great. Look at the pathways. And the mode of action is sufficient for a risk assessment because if we did not have that we would have to go to

default. And it can be a particular mode of action; I do not mean to do this negatively. My first question to the person would be, what is your background? Is it sufficient enough to help? I need to know where you are coming from. To help me evaluate your information. And I think peer review is necessary for determination. And with that, I think you very much. I appreciate your time. And given the opportunity to talk to you.

Audience Question: I want to clarify Michael. Maybe I've missed understood you. I wanted clarification about the paradigm for linearity in the report. There are two types. All of the individuals in the population at low dose might see some kind of low-dose linearity. It could be due to mutation. And that is one top of linearity. And another type, is where do you have various dose response relationship for the population and the individuals in the population. And a variety of individuals might be very close to having a response. In some individuals, might have a response because they are exposed above the threshold. In a way that is where you have variation in the thresholds within the population with the linearity dose response. Mike, I think you talked about both kinds in your presentation.

Dourson: That is a good point. If we are talking linearity with the Y axis and X axis and for cancer it is the dose response. If the response is [Indiscernible] the more chemical you give, the more the population will respond. There is a chance that could only be one molecule. But there is no threshold. In the case of cancer if their response is not [Indiscernible] or variability. The low dose behavior is the result of the individual dose response. In that case, if the dose response is due to variability, then the variability is already accounted for in the low dose response. And we have argued about this for a long time. The other idea that you mention, and Lauren this goes back to linear are not linear, but it goes back to thresholds. If each individual has a threshold, which for some responses we do. It could be a dozen chemicals that have a threshold. And you can write some of them off. In that particular case, the individual threshold for the individual test then the population has a threshold by definition. But then, do we have some exposure where people exceed the threshold? And that is likely to be true. This is not a threshold question but an exposure question. And that is a very important risk management determination for the dose response. You need to find the curve, first. I do not know if that muddles the water?

Zeise: With public health point of view, you have a good fraction of the population that is exposed to near threshold level overall. And you are contemplating adding and exposure to it. And you want to account for that. Whether that particular chemical that you are looking at, has an effect on the population. So, it just depends on the perspective you are taking when you look at the individual chemical. Are you looking at it overall? Or are you trying to determine the general sense? Or are you trying to make a regulatory decision or not [Indiscernible-- multiple speakers] because you have two different ways of thinking about it.

Dourson: I agree with you completely. If we have an exposure to chemicals, that risk management decision has to be contemplated. But of course, what is the foundation of that thinking is that each individual chemical that has a mode of action that is consistent with the threshold needs to be determined. People are exposed to multiple chemicals so therefore there is no threshold. But there are guidelines that [Indiscernible] look at it. But the mixture guidelines, predisposes that each individual chemical is a piece of that. I do not think we are disagreeing.

Zeise: I think it is just another dimension. I think there is the formulation of AOP, thinking how the system might respond to traditional chemicals. It can go beyond any particular mixture. But thinking in general of all of the different exposures that might affect that pathway. It can be endogenous it can be compounds, we have a whole host of exogenous exposures. And you

might want to make a decision on an additional chemical. And we can consider that in total rather than just looking at a mixture of chemicals.

Dourson: I think it does, but I want to clarify. Let's say you use EPA mixture guidelines, and they define things like what sufficiently similar means. Here is the activity of the individual chemical. And if you take and what that necessitates, your understanding of the individual dose response behavior, chemical and response. You cannot go to the individual chemical that is part of the mixture and say, I am going to predispose at that [Indiscernible] or other chemicals hinging on that particular chemical reference. Why? Because that mixture guidelines demands that you do not do that. It is having that chemical all by itself. Then you have a mixture exposure. All of those things that you are saying are absolutely correct.

Faustman: This is Elaine, I do appreciate having this discussion because it is addressing some of these questions. I think we can see it already starting to illustrate this. Go to slide number 23. This is for the examples on the conceptual model. I want to know, was this deliberate by the panel to put all of the individual dose response relationship with parallel slopes? Or was that implied that there would be? For the mode of action across individuals? And to handle variability. But I am wondering did the panel considered there might be variability in modes of action operational in individuals perhaps due to not only to background exposure but [Indiscernible] disease state?

Zeise: I think the figure was simplistic example. Just to enable the reader to see, how the background might integrate with the threshold. But you are absolutely right. Chemicals have a variety of dose response or biological activity. And there could be multiple kinds of activity for that particular toxicological process. And yes, people can be exposed to a variety that affect the pathways. You would not necessarily assume that all of those lines will be parallel. In fact, you are exactly right.

Faustman: Excellent. In my mind when I start thinking about AOP versus MOA and some of them we want to prevent in appropriate exposure to chemicals. And perhaps this is where that discussion should come from.

Audience Question: This question is from Bill Bracken. He says is it possible to apply these concepts to risk assessments for chemicals that have very little information available? In particular I was thinking about occupational exposure in a manufacturing environment and the need to set exposure limits.

Schoeny: This is Rita, and I would love to just mention that we are going to talk about some of these things in the later part, but one of the pieces that brings true to me for AOP is that they are established for a pathway and for a way for any chemical to be able to perturbed that pathway it an automatic and once AOP are well established and they are probably not right now, we are still in the beginning of this process, then I think they can be applied for chemicals for which there are data or for which there is a reasonable hypothesis that they could be acting through a particular AOP. Forgive me for speaking out of turn, guys, but just wanted to get that out as part of our discussion.

Faustman: I would agree with Rita. I think it is an issue of the individual regulatory body, or if it is occupational exposures, it could even be in terms of a particular business set or industry that is trying to assess the effects of the chemical under the globally harmonized system. And affect, in very sparse information, I think you can be kind of stock in the sense of you are blessed with default approaches. There could be similar chemicals where you understand a lot about their

particular mode of action and AOP or in the future potentially break you might do some read across. There is varying levels of lack of information and I think, Rita, you might even be thinking about cases where for a similar chemical you might understand a lot about how they could the acting and here along comes this other chemical where if you just look at the database for that specific chemical you are extremely limited, but in a broader context you might have a better understanding and you might even have some key informatics that you can basically use in trying to understand this additional chemical.

Dourson: Bill, this is Mike Dourson. I wanted to add a little bit to both what Rita and Elaine said. You don't have a lot of information so the next question you ask is what a problem formulation? Are you looking for an occupational exposure limit or some more quantitative aspects? If you are looking for something like an occupational exposure limit you know that is undergoing a transformation as to a quantification of safe dose or OEL. They are using vectors now and there are some publications that have come out that are assisting in that endeavor, because as you probably know better than many of us, there is a lot of variation in what's OEL is fixated around the world. Here is the good news. There is a framework that the WHO EFSA, the European food safety Authority has put out, and this framework is in one sense chemical specific. You can put your chemical into this framework and then you can come out with a threshold of toxicological concern. This number that you get is extremely low. Depending on your chemical structure, you can get different numbers that might be higher and if you have even more information you can take what Lauren has said about the types of read-across information that you can use, but the good news is this threshold of toxicological concern has been through international vetting, and it is actually going to be put out final any time now. It might actually be out final. I could be wrong. At least you have thought as a basis. If you are problem formulation is to get to an OEL you could get one now. Would probably be extremely low but at for data and it is likely to go up. I don't know if any of these answers got to your question. Hopefully they did.

Faustman: This is Elaine. I might ask Bill how much they are using read-across approaches for setting related OEL for some of their compounds that are data for. [Audience with question no longer available] You may not be able to come back and have dialogue. This is sort of one-way dialogue. We are seeing quite a bit being done by groups of compounds that has been assembled because of reach activities and some arguments being made that then indicate in poor cases can use a similar OEL. I think there are several approaches on this but depending I think Mike's point about what the exact problem is, but I think in the occupational setting it is to protect workers, obviously. Okay. We can't see the questions so can we have another one before we break?

Audience Question: This question is from Tessa. I just wanted to ask in the example of formaldehyde, point weighted the adverse events [Inaudible] of regulation would be identified as a point of departure? This is important because we have many chemicals which might induce enzyme of regulation but not proliferation or other types of tipping point events.

Dourson: That's a great question. This is Mike, and I guess to get the right answer, you would probably have to go to someone like Mel Anderson, but Mel Anderson's work, as I understood it, when he tested less than 1 ppm like .8, there was no activity in the nucleus. Now, you might say as a biologist, why is that? And I think the answer is because the cell had enough protective enzymes in the cytosol that they easily took care of the influx. Again, you need Mel Anderson here for the definitive answer. When you went to two PPM, you upregulated additional protective enzymes. That seems to me to be adaptation. This is kind of going down the NAS framework figure that we talked about before. You get adaptation, you get additional protective enzymes, and the cell can handle it. Point where the cell is telling you it’s hurt I think was the

five ppm where what got regulated? Repair enzymes. The cell was telling you it has to repair damage. That to me is the tipping point for formaldehyde. Was that five ppm or two? You can go to the actual data. The thing is which is nice about systems biology news and especially if you understand the underlying AOP you can query the cell. The cell will tell you when it's damaged. Apoptosis is certainly, what the cell is telling you is that the cell is not telling you, it is telling that neighboring cells I have so much damage I cannot repair it so I am doing cell suicide. I think you can query done and get tipping points, but I would really value Lauren and Elaine and other thoughts on the. Rita, you looked at this too.

Schoeny: I think it's a question that we have been wrestling with certainly since the first day I walked into EPA which was about if we are going to structure point of departure on an adverse effect, what do we mean by adverse? How at first is adverse? What kind of things are looking at? I think we are in a rapidly evolving place right now in terms of determining what is appropriate to choose as a tipping point or as an effect that would lead us to a point of departure. I don't think it is going to be the same for all chemicals and all effects that we are trying to avoid.

Zeise: Just to add, it is critically Santhosh people are they made up of a variety of different cells, of course, and cell systems alone are kind of limited at this point in their ability to incorporate how does their ability to reflect exactly what might be happening *in vivo*. Different individual cells differ, so when we think about in individual study looking at one cell line derived from potentially one individual, it raises concerns with respect to how well that is representative of what might happen in an *in vivo* exposure across a population of individuals. It is kind of a caveat also around inquiries that are looking for a particular single exposure and a limited *in vitro* system. Just a caveat around Mike's answer.

Faustman: I am hearing that a systems biology-based approach might want to be done before one makes it takes an action on an individual self-response in these processes. I think we are close enough to the break time. It is seven It is 712 and we're supposed to break at 7:15. I think we will reserve the three minutes to regroup. I'm going to declare success. This has been great. If we knew we would have such great lively discussion already before we had even gotten to Rita's talk and Craig's talk. Please return in half an hour.

[Break]

I think I am hearing that the audience is ready, so why don't we go ahead and reconvene. We want to welcome everyone back to the [Inaudible] as you know this is on the emerging toxicological science challenges and food and ingredient safety with a special emphasis on mode of action and those responsive actions in carcinogens. After having two very successful talks this morning, we want to welcome Rita Schoeny and she has been recently retired from the notion in 2015 after 30 years and is currently serving as a consultant on risk assessment and genetic toxicology. Her most recent positions at EPA were as senior science advisor in the office of science policy office of research and development and as a director of the risk assessment for the EPA office of the science advisor. She has 70+ publications in the scientific literature and tremendous amount of experience working with the documents in the area of ambient water quality, frameworks for risk assessment to inform decision-making and the list goes on and on. We are very pleased to invite retest to come and talk with us today, and in particular the topic that she is going to deal with is the mutagenic mode of action and doses for response analysis. Take it away.

10:25 am–11:05 am The Mutagenic Mode of Action and the Choices for the Dose-Response Analysis—Rita Schoeny, US EPA (retired), Washington, DC

Excuse me. This is Rita, and I am looking on the screen and let's see if my slides are up. Could you go to slide number two which should be the title slide and there it is. Let's move on from that, but I think the mutagenic mode of action is one that has a lot of interesting ideas attached to it, so it makes perfect sense for us to be talking about that particular mode of action or [Inaudible] pathway in this series of discussions. My conflict of interest statement is up on the screen at this point, and I really don't have any. Furthermore, I am no longer from the government that I am still here to help you in my capacity as a private consultant. Very importantly I do want to point out that what I am saying today are my opinions unless otherwise stated. I am not representing EPA or any other part of federal government in my talk. When I popped into saying we, referring to EPA, forgive me. I have only recently retired. The next slide is on policy, and it says EPA policy could be anyone's policy. We were strictly [Inaudible] against talking about policy in this colloquia, and these are scientific discussions, but I think we all hope that in the best of worlds science informs policy, and in particular it informs science policy which is something that certainly regulators and risk assessors lean on in order to be able to proceed with reasonable assessment went there are methodology adapts. As there are always data or methodology gaps, we need to have reasonable science policy. Next slide please.

One of the five policies articulated worth the 2005 cancer guidelines. I still think that was a major publication in that it changed a lot of our thinking in terms of what our defaults, how should we proceed, which I would be looking for instead of defaults, and I think it would us to a lot of useful discussion and certainly useful demonstrations of mode of action. I think it is now pretty much dogma that we as risk assessors and scientists and toxicologists care about mode of action, but what is on this particularly rather dated slide now is why should we care about mode of action? In truth we know now that mode of action is key in helping us with hazard identification, describing the circumstances under which an agent is carcinogenic whether this is specifically a high dose effect or root specific or if there are some other ways in which the animal data may or may not be useful for extrapolating mode of action is extremely important in determining whether the data set that we have is relevant for making choices about human risk. We have been talking this morning about mode of action helps us certainly in making choices about Lotus extrapolation, and the third button on here is to call to everyone's attention that in the 2005 cancer guidelines and in the supplemental guidance on life stage risk it was articulated that for a particular mode of action, that is a mutagenic mode of action for cancer that we should take particular care to ensure that people exposed in the early stages of life are sufficiently, quote, protected by the risk assessment that we put together. I think since that time we have become aware of risk assessment of a number of other areas of accessibility, but life stage risk was very much at the center of the discussion back in 2005.

If you would move to slide number six, and I believe there is some clicking that one has to do, thank you. What EPA's supplements until guidance on cancer said at that point was in any situation where the data are -- and methodologies permit, we should use age-specific values for both exposure and for potency for quantitative risk estimate of the chemicals for which we are evaluating. The splendid data permits -- for childhood exposure. Again, thinking more along the lines of 2016, one could speak into apply those cautions to other types of susceptibility as well, but let's just talk right now about life stage. It was decided back in 2005 that there were some data, and a lot of theory that indicated that if a chemical had a mutagenic mode of action there was increased risk when the exposure happened at early life stage, and also [Inaudible] but EPA chose not to go there all the other agencies did. As you can see in red, the default that was

articulated at that time is what one is doing the risk characterization and you decide you have a mutagenic mode of action or particular carcinogen, you increase the risk. Multiply the slope factor by default factors we call age-dependent adjustment factors. If you are looking at a population that is less than two years old, you take the slope factor and multiply it by 10-fold. If your population is between the ages of two and 16 multiplied by threefold. If you click once more, if there is no mode of action determined, when you do you are extrapolation, you don't apply the age-dependent adjustment factors, and obviously if you are doing a nonlinear, you don't apply the ADAF. What we said back in 2005 was don't worry, we have other additional guidance as time goes by. The EU for example back in 2005 and up to almost now, I believe, makes a distinction between genotoxic and non-genotoxic rather than EPA's choice to say we are looking at a mutagenic mode of action rather than eight genotoxic chemicals. Several US environmental agencies -- my screen just went dead. The fun of remote presentation.

Several US environmental agencies, on the other hand, prefer to apply some sort of age-dependent adjustment factor to all carcinogens rather than those that have a mutagenic mode of action. Nevertheless, if you click once more, EPA decided it might be a good idea to come up with a framework for determining a mutagenic mode of action for carcinogenicity given the fact that there is a lot riding on this particular choice or determination of a mode of action. A long time ago and risk assessment form group was put together to devise just such a framework. This was published as a draft in 2008 and there was public comment and peer review all of which was completed around 2008, and there have been several revisions of this document, but don't look for it online. It hasn't been published yet. However, I would like to go through some of the major points that were made in the revised draft, these have been presented at scientific meetings, and I think there are some very useful pieces that we came across in our discussions of mutagenic mode of action and what burden of proof when it needs to demonstrate that a chemical has a mutagenic mode of action. If you would move to the next slide, please.

Here are some of the major points of the latest draft which I'm sorry you have not seen on the mutagenic mode of action framework. A big one is that as far as the EPA guidelines read, and as far as other guidelines like PCS read, there is no default mode of action. When either is able to demonstrate that there are sufficient data to say this hypothetical mode of action is supported or not, EPA, and I think other agencies as well instituted some default procedures to follow when they are using now mode of action determined. As you probably know rather well, for EPA, the default when there was a mode of action demonstrable was to go back to the linear low dose extrapolation. That is different from saying all [Inaudible] mode of action. With you click again please. One of the big pieces that the various drafts of this document dead was to define genotoxic and mutagenic. Next slide please.

These are the definitions that are in the 2013 version of this particular document, and these were formulated based on consideration of a lot of sources, the EU in 1996 [Inaudible] in 2008 and carry Deerfield in 2002. All of these documents point out that there is a difference between genotoxicity and mutagenicity which is on the slide here. The biggest take-home point here is that mutagenicity is defined as the induction of permanent and transmissible changes in the DNA, in its chemical properties, its structure, the amount, this can involve those involved big pieces of DNA, a single gene or block of genes or base. But if it is permanent and transmissible, it is a genotoxicity by contrast is an alteration to the genetic material. This may not be permanent or trance miscible. The genotoxicity is a broader term. Again, the big point is the genotoxicity campy reversible, mutagenicity in itself is not reversible. Next slide please.

Here are some of the other points that we made a mutagenic mode of action like any other mode of action is a weight of evidence judgment and there is a hierarchy of data preference,

which again looks very much like other types of weight of evidence determinations that have been published by EPA and other groups. Generally speaking, they follow these sorts of dichotomies that I have listed here. Human data are preferred to animal data. *In vivo* data are preferred to *in vitro* data. It is much preferable to have data from the target site, and in this case, we're talking about cancer, so we are talking about the site of tumors rather than from another site as an indicator. And for EPA and for other groups that make the distinction between -- genotoxicity the preferable data are those on mutation [Inaudible] rather than other kinds of tests. Next slide, please.

This is one articulation of the data preference that is in various formulations of this framework for mutagenic mode of action and this is based on some work by Martha Moore, and they are just logical. The best data to indicate that a chemical has a mutagenic mode of action is a demonstration in a cancer relevant oncogene or tumor suppressant gene that you can attribute to the particular chemical in the target tissue following chemical exposure. Obviously, those data are few and far between. Thus, there are other data that are high in the hierarchy such as a surrogate gene mutation or chromosome elaboration in the target tissue following chemical exposure. There are assays now [Inaudible] and so forth that allow us to look at the target tissue in an animal following chemical exposure. Goes down the list. Chemical specific adducts in the target tissue. Primary DNA damage in the target tissue. Gene mutation in chromosome after *in vivo* and then you finally get down to what we most often have which is evidence from an *in vitro* assay that the chemical causes mutation or cytogenetic damage or DNA adduct or other kind of primary DNA damage. That is the hierarchy. The next slide please, shows some of the steps in the weight of evidence procedure, in the first one obviously [Inaudible] was assemble the relevant data, and in this particular instance, and I think for any mode of action work, the relevant data are not limited just to those assays that measure, for instance, mutation. We would assume if we are looking at determining a mode of action that we already have some indication that the chemical is carcinogenic, either tumor data on itself or reasonable data from a read-across or [Inaudible] or what have you, but there are plenty of other datatypes that need to be examined and assembled before we would make a choice about whether mode of action is supported. That is going to include pharmacokinetic data [Inaudible] biochemistry, looking at other potential modes of action and so forth.

Step number 2 I would point out as being particularly important when looking at genotoxicity data. Evaluate the data in its current acceptance and quality criteria. Genotoxic data were and still are rather inexpensive to generate [Inaudible] doing *in vivo* work, and quite frankly there has been a proliferation of genotoxic data of various sorts [Inaudible] the typical -- it has been done a lot on a lot of chemicals. It has been done on one chemical multiple times, and quite frankly not all of the data are as good when one begins to evaluate. There are good guidelines that have been developed by organizations such as OECD and the international working group on genetic toxicity data, and they are still being developed. Quite frankly OECD has just published revised guidelines on a number of genotoxicity assays, and a big focus of this has been how to interpret the data. How do you ascertain whether you have a positive, a real positive, in undeniable negative, or something in between? How do you evaluate these data? It is not as cut and dry, perhaps, as pluses and minuses in a box. The data need to be evaluated again. Current acceptance and current quality criteria and interpreted under those types of guidelines. Then you get down to what we are mostly involved with in terms of risk assessment and doing mode of action. Apply your guidelines. EPA cancer guidelines, IPCS guidelines, OECD guidance for determining an AOP, one of those things.

Among the lessons here is that in applying these guidelines, one discovers rapidly that genotoxic is not the same as mutagenic. Not by definition. Something that is mutagenic *in vitro*,

for instance, may not be mutagenic in humans or in animals, and something that is absolutely mutagenic in a number of different tests may not have a mutagenic mode of action. What we are saying here is that again one has to go through the usual rigor of evaluation of individual assays and weight of evidence to determine a mutagenic mode of action that you would have to do for any other kind of mode of action determination.

Slide number 13. This one has been around the block a few times. Martha Moore has had a handedness and so has Tera, but the point is that these tests measure different types of events. What are listed here are three tests that actually do measure mutation. Chromosome aberrations in CHO cells and Ames test, salmonella bacteria mutagenicity test for mutation aspects. See, even these measure different types of events. Ames test measures do not. Ames test does not measure any kind of chromosomal damage [Inaudible] to answer this [Inaudible]. It needs to be pointed out here that there is a lot of DNA interaction measured in genotoxicity tests that are not on this chart. For example, DNA adduct do not measure mutation. Mutation tests may or may not reflect DNA adducts. Next slide please.

What we are getting into here now is the DNA damage is not the same as mutation. Mutation is not the same as cancer. This is leading to some of the final conclusions that mutation is not an absolutely instantaneous event, nor is cancer. There are various steps that take place. What we're looking at here are some very old, but revealing data, and some newer data. The lower left here on this chart is the mega mouse study which we are all familiar with which indicated to us that it certainly looked for this particular person [Inaudible] and a lot of mice, that liver cancer was induced with a dose responsive, certainly looked linear at what is what is where as the bladder tuner aptly did not. Later on, one began to look at DNA adducts which largely looked linear again, but not all DNA adducts are the same. I would point you to a number of papers that grew out of a [Inaudible] project on how to evaluate DNA adducts data in the context diversity assessment and [Inaudible] and that was one of our major points. DNA damage, DNA adducts are not always the same. Some are mutagenic, some are not.

Building on the statement that not all data should be weighed the same in terms of genotoxicity, let me draw your attention to the next slide. This is slide number 15. What this shows is an array of genotoxicity data in a very ingenious fashion. It was devised by Mike Waters and his colleagues at NIEHS and was used as the system was put together for a number of years. These are called gaps for genetic activity profiles, and what this does is an array of data on one relatively simple side. What you are looking at our data from [Inaudible] lower left-hand corner all the way through human data. These are individual assay results, and what you see is a horizontal line drawn through at zero. This separates positive, above the line, from negative, below the line. It is a beautifully done reverse log dose. So, above the line, the higher or longer the line, the more potent chemical. That is the positive response at a lower dose whereas below the line what you are looking at is highest dose tested that gave no response. [Inaudible] pharmaceutical agent, cancer therapeutic agent which is certainly carcinogenic in humans, and it will mutate just about anything that you present to it. For Cyclophosphamide, you have a large weight of evidence that it is mutagenic. It just blows your DNA apart, and it will cause mutations in just about every system in which it has been tested.

By contrast, take a look at slide number 16 and this is for chloroform. This has been the EPA poster child for a carcinogen that does not act by a mutagenic mode of action, but look on the gap, the genetic activity profile, there are some positives. One cannot just add up all the pluses and all of the negatives or take for example that one has a positive in any test, and that indicates that something is mutagenic. One has to go back, and you can do that with these gaps or with other types of data arrays and look at where the positives were found and under what

kinds of circumstances. Again, predominantly negative responses, but if one goes to the few instances, and there are some [Inaudible] *in vivo* test above the line, you have to look at those tests and see where they interpreted appropriately. Is this something that really is indicative of mutagenicity? I'm going to ask you to trust me that on chloroform these were not tests that were indicative of mutagenicity or were inappropriately done. Next slide please.

What we are looking at is any kind of mode of action determination is a weight of evidence versus a strength of evidence determination. That is to say that one positive does not outweigh a number of negatives, and one goes back and looks at what these data are telling us rather than as of the pluses and minuses. Dave Eastman has done some very elegant work, and here note that we switched from chloroform to carbon tetrachloride, but he and his colleagues went through and took a look at all of the data for carbon tests that were indicative of the genotoxic effect and took a very systematic and objective way of looking at all of these. What you see, for example, these are *in vivo*, in these our data that are quite substantial in terms of determining whether something is actually mutagenic or not. What we see for carbon tests is on the right-hand side a fair amount of data is that it generates reactive oxygen species and can do something that would damage DNA in that sense. At what dose, that is an important question. Mutations *in vivo* in mammals, what one has are no positives and three reproducible negatives. That is part of what would take into consideration in this type of weight of evidence. Next slide, number 18.

What we recommended in the various drafts of the framework was that one array the data in a systematic fashion and take a look at each of the studies, not eliminating anything but making note. For example, this study was done under a protocol that is no longer used. You put it on the charts, you evaluate it, you weigh it. You weigh the data for each of the various endpoints and make some stepwise choices and ultimately decide whether there are sufficient data to indicate that the chemical is mutagenic and likely to be mutagenic. Next slide. Then you apply a mode of action framework and as it has been pointed out before the EPA and IPCS frameworks are essentially the same and rely on the same kinds of steps, identifying the key event, looking at the experimental support, strength, consistency, specificity Association, dose response concordance, temporal relationship, and I think to most folks working at MOA and AOP, the big one is this is biologically plausible and does this make sense? Ultimately is it relevant to humans and are there other things going on and so forth. Next slide please. Number 20.

One of the big pieces that has been quite useful in determining whether something has a mutagenic mode of action is the dose and time concordance? These are some fly essentially from Martha Moore. On the left-hand side what you see here is what one would expect for something that has a mutagenic mode of action that is the blue line, you would expect to see mutations relatively quickly after a treatment, after a short treatment, after a treatment that has been stopped you may still see some mutation. On the other hand, the red dotted line, if you're looking at something that is likely to be non-mutagenic there may not be this sort of time concordance. You wouldn't expect. The hypothetical is on the right-hand side. If you are looking at a mutagenic carcinogen, one that has a mutagenic mode of action, that particular chemical initiates the mutation. That is how it gets the chain of events started from first exposure to adverse outcome. Initiating mutations, multiple events, and then a tumor. If you are looking at a non-mutagenic carcinogen you realize that there are mutations in cells all the time, but what is happening with your particular chemical is that it is either altering gene expression, it is enhancing cell proliferation, it is killing off cells of a certain sort, and what it is doing is providing the agility for initiated cells to either proliferate or survive rather than causing mutations themselves. Next slide.

What people are using now are dose and time concordance charts so that you can put together a lovely matrix showing what sort of events you would expect to have early on in your hypothesized mode of action or AOP and the type of data you would expect to substantiate that sort of event. For example, and I am sorry these are rather difficult to see here, but on the left we are showing an almost classic mutagenic mode of action or AOP for toxin AFB1. The first thing that has to happen is the aflatoxin is metabolized to reactive form which then combined with DNA you get a specific DNA adduct. This is not repaired or is repaired in an error-prone fashion, and you get fixation or mutation. That goes on to induce proliferative lesion, altered enzymes for example. Finally, you get to cellular carcinoma. You expect the early events to take place early, and at lower dose than you would expect the later events. Some of these data are available. By contrast, the mutations are almost irrelevant, and I will show you another slide along those lines. Lynn Pottinger and her colleagues are putting together a very elegant paper on the adverse outcome pathways that is demonstrated by polonium oxide and I will show another slide on that in just a second. Next slide please.

Mode of action, AOP. They are not the same, but they have some very similar uses. It is important to point out that the mode of action is specific to a chemical. The adverse outcomes although we have been using particular chemicals as case studies to try to figure out what is going on. They are chemically agnostic. The mode of action uses the modified criteria that are published by IPCS, by EPA, the AOP is now using the evolved criteria which gives more weight to some criteria than to others, but if you take a look at the little button, they have the same kinds of uses. And forming hazard ID, informing dose response, identifying life stage or other susceptibility in humans, and very importantly grouping chemicals from the exposures. We will talk about that some more when we get into our later discussion. Next slide please.

This one is showing a couple more big conclusions, and the reason why I bring up AOP here is I will show you one case on the conclusion that mutagenic or even genotoxic and even carcinogenic is not the same as having a mutagenic mode of action. One of the case studies that was published in Pottinger et all has to do with DNA adducts which are formed by Tamoxifen it is a cancer chemotherapeutic drug. It is carcinogenic and humans. It appears to cause endometrial tumors. It is carcinogenic in rats, and it's produces liver tumors in rats and it produces liver tumors in rats I believe. It is mutagenic, and actually causes DNA adducts in rats. But is probably not important in humans in terms of the types of tumors that are being produced. It is a hormonally active agent, and it appears that that mode of action is the way it is producing endometrial cancers in humans rather than through DNA binding. I have an unnamed pesticide on here. This is something that [Inaudible] discussed from time to time in discussions of mode of action. There is a nameless pesticide that is nicely mutagenic, produces methylated DNA. The only tumors it produces in any animal system study has been fibroid tumors by homeostasis by acting as a hormonally active agent, not by virtue of its [Inaudible]. If you click on more, this is a proposed adverse outcome pathway from study of propylene oxide. This is an adverse outcome pathway that is indicative of, reflects the data that have been collected on this chemical over many years. Lot of inhalation assays, three of them, a lot of mode of action work, and with the chemical does is to produce nasal tumors at certain doses and not in others. The proposed AOP for which there is substantial data is that the chemical completes GSH in the course of its metabolism and that starts the cascade of events moving along that ultimately leads to nasal tumors even though one can demonstrate under certain circumstances that propylene oxide is mutagenic, causes point mutations and cellular mutations as well, but the nasal tumor mode of action is complicated and it would appear that there is a practical threshold. Next slide.

There are plenty of data and theory that indicates that even if one has demonstrated a mutagenic mode of action, that does not necessarily mean what does low dose linear, certainly

not for all of the key events or key event relationships in an AOP or mode of action. This is from David Eastman that note some of the mechanisms that are associated with nonlinear dose response determination. For example, the first button, critical involvement of non-DNA targets such as repair enzymes, such as the mechanism involved with [Inaudible] formation. Note that the second button, DNA repair is extremely efficient in humans, in rats, even in bacteria thus contributing to what one would expect to be a nonlinear dose response or mutation induction. One think that we pointed out for carbon tests on the second column, indirect origin of damage can bring about the formation of reactive oxygen species, but that is very unlikely to have a linear type of kinetic. Next slide.

Mutation is not a one-step affair, and this is a very simplified mutagenesis paradigm. DNA is damaged by endogenous materials, spontaneously, just in the course of DNA replication, however one went up with damaged DNA. There are multiple and backup damaged systems in all organisms that have been studied which leads to a cellular response. The on the helplessness frequently is DNA repair. The DNA is repaired without change or one can show that there can be changes, but not those that are expressed. In some instances, fairly rarely, there is incorrect repair, there is subsequent replication of this mistake, it is expressed, and one have a mutation. In some situations, on the far right, there is no repair, or the repair is such that the cell can't survive in you have a dead sell. This is a bummer for the cell, but it may not have a major effect on the organism. Next slide. Stop right there. This is good. These are some data from Alexander long which were presented by Paul White at the recent meeting and [Inaudible] toxicologist in other toxicologist has been doing is taking a look at in extended dose range, particularly in the low dose range, because that is where we want to know what is going on. What this shows is the [Inaudible] muted frequency. This is *in vivo* test. Would you click one more please? These are the DNA adduct frequencies again measured in the same model, and they certainly look linear at first glance. Next slide please.

If you take a closer look at the low end of the dose response range, what you see is a somewhat different picture. That indicates just by looking at that the DNA adducts are once again low dose linear, but the frequency is not. It is not over a reasonable range between, I don't know what Alexander's absolute lowest dose was, but certainly between the lowest dose up to 3 mg per kilogram a day. You absolutely have a mutagenic response taking place. My colleagues who are highly invested in DNA adduct work wants me to mention that the type of adduct data being shown here as being low dose linear are not specifically pro-mutagenic adducts, but rather all adducts that are being tested. Again, one can look at the lovely publications for elegant discussion of what kind of adducts are most likely to be mutagenic and which ones are not. On slide 28, these are some data from N-ethyl-N-nitrosourea and one of the things that got the genetic toxicologist really looking for demonstration as to what is going on and what is exposure to mutagens was an incident some years ago when a pharmaceutical was contaminated with alkylating agents and the received wisdom on alkylating agents is these things are bad. They are carcinogenic, they are mutagenic, they are not likely to have any sort of safe dose but there was a population exposed to them alkylating agents, and it was incumbent on toxicologist and genetic toxicologists to investigate to see what kind of damage we could expect from this inadvertent exposure. There were a lot of studies done on alkylating agents, N-ethyl-N-nitrosourea and in early papers, these two chemicals showed a threshold for a mutagenic effect. ENU and MNU did not show thresholds. MMS did not have a threshold. When you go back and look at some of these things and apply more recent modeling procedures, you can get a lot more information as to what is going on at low dose. Later modeling by [Inaudible] and on edge by George Johnson showed that there was a break point, you can call it a threshold for [Inaudible] antigenicity. Next slide please.

Genetic toxicologists have been vehement for years on saying that mutation is not instantaneous, that one ought not assume certain types of dose response curve, but these arguments are beginning to get some traction and if – [We're just giving you a little warning about time. We are already over. Thank you, Rita.] One of the big points by HESI GTTC group, you get a lot more information than a guess or no. Mutation of itself is a toxicological relevant end point and one would want to do quantitation on this. Next please. You can determine a point of departure from *in vivo* gene tox data for use and quantitative risk assessment and there are a number of lovely publications by international working group on genetic toxicity, and the [Inaudible] group and we will click quickly through the next two. Keep moving. There are various definitions you can look at later or point of departure that can be calculated. Next slide. These all have advantages and disadvantages by and large the group is working on this as a benchmark dose as your preferred approach [Inaudible] and next slide a conceptual framework, keep clicking, is to determine a point of departure of some sort and not try to extrapolate below that, but rather look at uncertainty or safety factors or calculate margins of exposure and the assumption being that at low doses this is a reasonable assumption, there are still protection mechanisms that are highly efficient, not saturated, and you are not going to seem a response it is distinguishable from a spontaneous background. Next slide. I provide you some references to these IWGT papers and the very last slide acknowledging that there are a lot about people working in this area right now on mutagenic mode of action, on AOPs and [Inaudible] genetic toxicology. They do so much and sorry I missed the queue to be quiet.

Rita, thank you so much. That was a very great presentation. Thank you again for the references at the end. I think we are going to hold questions in the interest of time and move to Craig's presentation next. Please hold your questions. We want to come back and ask Rita. We want to come back to read assume. Let's hear from Craig. Doctor Craig Rowlands is a senior scientist at the Dow Chemical Company toxicology and environmental research and consulting organization and he directs their research program on environmental pollutants. He is also an adjunct professor at the Center for Integrative Toxicology at Michigan State University and prior to joining, Dow was a scientist at the US food and drug administration and an assistant professor in the department of pediatrics and pharmacology at the University of Arkansas. He is going to be those we want to welcome crack to the microphone to give us our last, but certainly not at least talk today. Please join us. Thank you.

11:05 am–11:45 am Risk21 Quantitative Key Events Dose-Response Framework—J. Craig Rowlands, Dow Chemical Company, Midlands, MI

I may be talking about a project that came out, the Risk21 project and [Inaudible] mode of action human relevance framework and I want to talk about two things. One is the Risk21 framework and that is additional framework which was our quantitative key events dose-response framework. I want to briefly revisit what is going on in the paradigm shift of risk assessment so we can tell you why we thought this project was necessary and then I will describe to you the HESI Risk Assessment framework in the 21st Century that was developed and then I will turn the attention over to the Q-KEDRF framework and hopefully highlight some points using two case studies if I have time for sure.

No problem. All I was trying to say is we heard about the three major reports talking about the toxicity in the 21st century, the science and decisions course and the main driver while we are here today [Inaudible] we heard Lauren give a very nice overview of that this morning. One we haven't talked about specifically is a companion to the 21st century toxicology report which is exposure science in 21st century. The toxicity test is really focusing on the hazard aspect of how to move into the 21st century with the new paradigm on determining how are predicting

hazards using largely electro cellular approaches were available in the last fifty years and biomedical research and how we apply this toxicology and also how do we get this into human cells and tissue and predict rather than do animal studies, largely *in vitro* approaches. Cellular science with a similar concept in the sense that we wanted to try to figure out how to you best use new ways of collecting chemical exposure information, how to do exposure modeling and for the challenge of how to keep up with the speed of doing *in vitro* toxicity testing so we had exposure information available to do risk assessment on Science and Decisions course is rethinking how we should be doing risk assessment and general bringing forth a lot of the information, knowledge, and experience that we have gathered over the twenty seven years and proposing adjustments to how we should be practicing risk assessment. Next slide.

The major foundation of all of this 21st century toxicology risk assessment is mode of action. Mode of action is really at the center of all of the proposed methodologies for doing *in vitro* risk assessment and also for doing what we are talking about today is using key events for dose response modeling. One point just makes my assessment between the difference between mode of action and AOP is the way I look of it is mode of action is nested within AOP and you can't really build out of AOP without understanding mode of action. Because of all of these proposed changes we felt that it was time to pull together and figure out how we could reassess and propose a new way of doing risk assessment that would encompass many of these proposed changes and proposals in this report. This was the HESI Risk 21 program. Next slide.

Go ahead and built this out. There are several lines here. [Inaudible] multipart stakeholder groups that are built [Inaudible] Risk21 we were able to gather more than 120 scientists, largely from Europe and the United States and this has been developing a risk assessment approach embracing all these proposed changes and advances of and methods, ends we feel that the end product of currently revising how to approach the science and the art of risk assessment. Next slide.

This is an info graphic essentially of what the proposed Risk21 framework looks like. There are four steps to the framework starting with problem formulation and exposure and then toxicity and a tool that was created which was to visualize all the information has been gathered we call the matrix and that is the fourth step. You'll see there are inverted triangles for the exposure and the toxicity phases and the narrow tip of the triangle represents a little time and money and effort in the with each step you broaden the time and money and effort that is involved in gathering that information. The same is true from the toxicity information as well. Go ahead and go to the next slide.

This is the matrix. This is a new tool that was created for this project. This is an estimate exposure toxicity that allows a certain accuracy of precision and all the factors that would go into this matrix and allow a risk assessor or anyone else to visualize all of the information that is available. I will tell you a little bit about the details [Inaudible] scale it is where you have the y-axis at high toxicity leaving the lower numbers, so you are going from low toxicity to high on the y-axis. Your actual exposure levels or doses are higher towards the coordinate in the bottom corner in the low to high exposure estimates are on the X axis. These have to be in the same unit and part of the work has to be where all this thing is.

Once the information is gathered, then, it is put into this tool and then you can see very easily here this blue box of what is called risk box, and green at the lower left corner is of course safe levels of exposure up to the far right corner is the hazardous levels of exposures, high risk levels and the yellow represents the margin of exposure. That margin of exposure is changeable in the tool itself, and so I believe here it's set to one through 100, but that is where

risk assessor favor with the margin exposure in understand whether or not the exposures are acceptable based on what is available and also what this tool allows to identify [Inaudible] needed to understand the risk and reduce the uncertainty. That is a very fast overview of the matrix. I just wanted to get that out there. We could go to the next slide.

The first step is problem formulation. This is where you're going to identify the object is to scope out the project and develop a hypothesis. The hypothesis is created of from. Your you are asking what do you know already without having any more information? What does one need to know based on that? What are the gaps? And critically been how do you know when they are done? How do you know when they have the right amount of information? Of course, that is going to be a decision context answer depending on the regulatory decision, that'll change but that is a little more on the policy side than the science side, I would imagine. Go ahead and click.

The tagline of the project that we say the project is really identifying to see if you have enough precision to make a decision. No one wants to waste any resources and one wants to get to the answer as quickly as possible. How does one know when does that you have enough information for that position? The matrix I hope to visualize in being an iterative process allows one to get to that place where it is in a fairly rapid fashion. Next slide. I won't go through this for the interest of time, but when you look at this like you can see some more detail on examples of how much an increasing amount of information is going up the tiers. Click, please. We talked about approaches as a good place to start. That's really not very resource intensive pockets a good place to start a reading for us or detoxified concern that can go from there all the way to the very top. Which is very intensive research the resource of love what we're talking about today which is the mode of action and this is where the dose response or rather the framework was building off of, taken off of mode of action information and using that information and key events to help reduce a certain amount of risk assessment decisions. So, this is a web-based tool it is readily available to anyone. To go to www.risk21.org. You can find information, case studies, and anyone can put in their own information tools and begin to see those risk boxes created that I showed you earlier. So these have now been on five publications in critical reviews toxicology. There references are there and details about everything I just went through our presentation including two-page case examples- which I will be going through shortly.

So, to turn our attention now to two case studies. One is going to highlight how to use the Q-KEDRF framework to understand the margin of exposure. Information which is much more available visually and the second the study to use the approach to look at those response modeling of key events to various forms of final response does response model for toxicity when it should look like. So, here's our matrix again. There are four steps involved. We will go through this information now from a mode of action level. Again [Indiscernible] is building off the top docs. So, what you're looking at here is the different way of viewing the mode of action in the framework of the top left color, the green or blue and orange boxes up there. And this project was of that framework, Mode of Action/Human relevance framework and then extended it from the point of the mode of action is agreed-upon or known and it has the relevance that we are going to extend that into the Q-KEDREF framework. So, going down to the white box to start with the Q-KEDREF [Indiscernible] potentially that point. This is a series of [Indiscernible] that begin -- bet [Indiscernible - interference on phone line] on the key events and makes it available for those. To which is events can be identified unequivocally with associated events and we would talk to [Indiscernible - muffled speaker]. Going on to look at the dose response and the slope and the temporal relationship [Indiscernible] and incidentally were quantitative assessments were key events. And also increases the wave of evidence that substantiates the key events in the mode of action. This also then because it has questions about what we call

modulating factors. For the events that can impact the response for the toxicity. And by looking at the shape of those response curves for the key events and can help inform at the final does response looks like for the toxicity.

So, in short Q-KEDREF is a structured approach. The framework for the key events that has mode of action in the quantitative aspects of dose response. It does incorporate a couple new concepts/ We call them associated events and modulating factors. With the concepts which is giving new names and recently having name explosions here. We have a couple more we have to deal with. But I understand the distribution of population first so that sensitivity. Hopefully the adverse outcome for the response.

We know what the key event is. We talked about it, that it's necessary but not always sufficient. Associated events are currently biomarkers for key event. There the biological process again by themselves are not key events. They are not in the cargo pathway toxicity, but they're tightly associated with changes in the key events activity. So, enzymes for example, are great associated events to a key event. Modulating factors are all things we talked about this morning. These biological and individual factors that impact [Indiscernible] and the magnitude ultimately of the outcome that to the adverse outcome. So, there's two different concepts, both very important to understand as you work through mode of action framework.

So, we find these modulating factors both of the human organism level and cellular level. Think this event genetic and [Indiscernible - muffled speaker] more prisons and lifestyle factor that you take into consideration when doing the modeling. With environmental factors of course and other mixtures etc. that they might live in. So, for 21st century toxicology at the cellular levels. The *in vitro* assays of the cellular environment itself. But that will impact dose responses. And that may work for the structure gene to mutations, polymorphisms and also gene expression is at least 100 proteins needed to express genes. Means is pretty much opportunity for different levels and dose response modeling. And also, the cells that constitute cellular phenotype that you are look at. This can be modified. And many others you have to just to give a sense of what is considered for dose response *in vitro* data.

So, the example I want to use and finalize the margin of exposure aspect of the Q-KEDREF tool is Dimethylarsinic Acid. So, this is the mode of action for DMA. It is metabolized to trivalent DMA-3. In the bladder tumor origin. In the toxicity of layer of the urinary bladder. Which then leads to tissue repair and [Indiscernible]. When this occurs in excess will lead to the hyperplasia and if it's sustained a long enough period time, this is critical must be sustained for very long. Time. This within allow for some of the hyperplastic cells to transform a tumor cells and week can have bladder tumors.

This is what we had talked about with the dose-time concordance table. This is the mode of action framework. It is a nice tool [Indiscernible] with all the data related to the events across the top of the columns are time, it's temporal information and going down the road and increasing dose. And then bring up the very quadrant of the table to identify where the sufficient dose and sufficient time for the important key events here. And so [Indiscernible] for example unless the dose is 40 (I believe this is PCT if I am not mistaken. And doesn't occur until 104 weeks. Even though all the key events are observed at 40 [Indiscernible]. And of course, at 100. If you go below that dose to 10, we don't see the very important proliferation and hyperplasia that leads to cancer. So [Indiscernible] see it's easy to see the relationship of the time it is here.

This is a new table that we created but really give you the relevant comparison and essentially what it is doing is going on animal beta right next to human data for each of the key events. So,

if we go down the table to key event number three the very bottom row, just as an example, when can say that there is observed [Indiscernible] perforation for DMA [Indiscernible] and there is no data available in humans for this effect. Courses are probably common for this course of action. Because of metabolism and biology really suggest that this is possible in humans, you cannot rule it out. And then there is the quantitative concordance next to that and that is the dose response for the actual animal data. And if there was human data available and look at dose response in the human data side-by-side. So, it's just a systematic comparison across humans an experimental model animal. There were some key events I laid out there. So, this is now finding that information onto the matrix. Example how this can be used to understand whether or not humans are at riskier or the doses are exposures are not likely to be cancer. So, the mode of action is color-coded. The toxicity is where you see the blue triangle on the matrix. In the triangle is the low-fat level that key event and that whole range of the triangle to the bar, they estimated human exposures.

So, then the second key event is a much higher dose. That is the regenerative proliferation and the same exposure levels. So, you don’t get cancer until you get a third key event for sustained period of time that takes a higher dose and then the terms occur even higher for the dose. So, looking at that information is pretty visually easy to see that looks like right now because it's on the green at least for the two key events for example, plasia or and hyperplasia. That may or may not be sufficient to penny on the regulatory decision. And whatever decision context this is in to. But it is very easy to look at the dating back to the tool now and adjust that margin of exposure to you can see that yellow band shift up or down depending on what is at the margin of exposure to give you a sense of whether or not you're likely to produce all the key events so events must occur to produce a tumor and whether or not human exposure it asked [Indiscernible] over the range for the key event. That's what easy way to share one with the tool makes it easy to visualize. It also allows one to go back in and gather more information if it looks like there may be a problem and the exposure could be tightened up by looking at the exposure collection. Remodeling or even mining for data. And so rather going to the animal studies for the effort might be tested exposure information. And sometimes it can be very easily visualized.

So, for the mode of action around this margin exposure example, the Q-KEDREF, tool itself allows high-quality dose response data key events and apical events. You need this information or to use the tool. The key events can be adequately identified such. Using the tool, the position and the steepness of the dose response curve should be considered, and I do that better on the next example. I think I have time to go through that.

The modulating factor account relative to the dose level of interest that can shift the dose response. It’s more qualitative than quantitative. It is difficult to anticipate the or actually measure the effect of each module infector on a dose response. Certainly, of the organism level and in humans themselves. Certainly, one can hypothesize how it would impact and try to factor that into any sort of risk assessment. In certain factors. The quantitative dose response gives the key events pretty much keep the mode of action as well. By doing this we can go back and provide additional evidence about the soundness of once proposed mode of actions.

So, the second example and I am sorry to go through the so fast, but I want to make sure I get through them. This is the dosage model using the AH receptor rat liver tumor promoted mode of action. This is one of the key case studies that within the Q-KEDRF manuscript. But this is done using the Q-KEDREF process. So, it is actually applying this beyond this. So, this AHR mediated rodent tumor liver tumor promotion has been extensively studied and a consensus mode of action that has been published a couple years ago. This case highlights concept of impact dose-response that would include the modulating factors and the associated events, and

also the final modeling feature key events that helps in the dose response model for the tumor response.

So, I have to give you a little bit of background. The Aryl Hydrocarbon Receptor course is a ligand-activated transcription factor, although it doesn't belong to the same family. It was activated by a ligand analysis. Finding the partner protein and approaching that nucleus [Indiscernible] and modulates up-and-down. This will lead to changes in protein expressions that answer much of it affects that are observed in the multiple lists of toxicity that are in animal studies. It is a very promoted receptor motivated by a large number of chemicals. That study in that carbons of the [Indiscernible] TC's most potent. Move the carbons will reactivate the receptors as well. And interestingly a large number of plant chemicals in the planetoids and call the [Indiscernible] have been published over the years. But even in multiple [Indiscernible] it is really not known still what endogenous ligands for the age scepter if one exists at all. It does regulate what you said a number of genes up-and-down. One way to divide pieces into the phase 1, phase 2 metabolic enzymes which are really related to the adaptive changes. Metabolizing chemicals. And others related to some of the toxicity. Example one is one called Tiparp and actually Tiparp is now known to regulate the age receptor itself and also the sensitivity dioxin. Very interesting observation.

So, mode of action for liver tumor promotion shown here. So, we start with the articular ligands. and then this would cause sustained activation of the age receptor. Which would then lead to cellular response changes and for the age receptor we know the cost changes in a proptosis. Of cellular phosphatases. This then leads to the organ response to the pair toxicity the term called hepatopathy. This is where it captures a lot under one name. And also, hyperplasia. And if you organism response of course can produce liver tumors if this is sustained for a long enough time. So again, here is the dose [Audio cutting out] table and what is showing here is across the top we have the time-dependent changes and key events going down the left, the dose, increasing dose. And we don't begin to see any key event changes of consequence to the dose of 1000 [Indiscernible] [Audio cutting out] liver concentration as they expressed here. 2000 mg are all critical key events observed that would then lead to cancer in this case the first two tumors a sheet of two years. The second type of tumor that is observed is the carcinomas and that takes a dose of 5000 mg kilogram to produce the final [Indiscernible]. So, you can see the relationship of the dose and the time and even the type. [Indiscernible]

Here's an example of using the dose response for the key events to demonstrate first of all the key events that it looks like it is very closely related to work evidence of this is it [Indiscernible] with the dioxin. And [Indiscernible] 31 weeks. The events it did not develop toxic hepatoxicity and there were no liver tumors. And what you're looking at here is a very nice example of a case information to shore up the idea. Which is not necessarily key event because without it you don’t get the tumors. And with it you get very clear tumors themselves. And this is very close to the longitudinal formation as well, so the dose response actually tend to mimic toxicity because you get to closer to the actual physiological change [Indiscernible] at the tumor level.

The next slide is where I put on here all the dose response information that was very detailed for the different events across the AOP. Starting with the first sustained AHR activation. Here it has the coefficient of one, and you’ll see that the [Indiscernible] represents will eventually change becoming a higher [Indiscernible] which indicates the steeper slope. And moving over to the cellular [Indiscernible] are now three dose response occurs in that box or on the figure. But now we also have time-dependent changes. Going for one month to six months. And it is not until the six months that we begin to see a nice dose response for the changes in this case it is actually [Indiscernible] inhibition. And [Indiscernible] [Indiscernible] independent. The current beginning

to change shape and once we get to the organ response level [Indiscernible] and hyperplasia. To take a look at the center one for example there are no changes at 53 weeks. But at 104 weeks there's a clearer dose response increase in oval cell hyperplasia. Services does it time-dependent. Clearly now the dose response begins to look more like [Indiscernible] response well. [Audio cutting out] over to the far right and you have the carcinomas on the top and adenomas on the bottom. And so, as we have more biology we begin to see steepness of the dose response increasing and so that is indicative of probably the complexity of the biology that second-place. The point of departure for those issues to the right further along the complexity and dysregulation that we are seeing across the key events. So, the modulating factor associated events are useful in describing the nuances of this mode of action. Modulate factors is working altered that occurrence of one or more [Indiscernible] and the associated events of the indicator for the key event. The beginning of itself is not a key event but it's a very good marker of key event changes.

In our example, we use the associated events as Desyrel enzyme activity which is a sip 101 gene coded for neuron activity. And to this is always very tightly coupled expression with interceptor. So, we're using indirect measures associated events. In addition, under some circumstances or some mode of actions have also suggested when the same enzyme system can actually be a modulating factor for example should think of estrogens in female rats, you could actually make case that that is also something that needs to be in consideration as perhaps a modulating factor increasing the sensitivity of animals in the female rat.

So, the MOA/HRF framework along with Q-KEDRF provides a strong foundation for using this information as a means to reduce and the risk assessment for additional tools for the to relate the key events together. And the adverse outcome in a quantitative way both those the time dimensions and the dose response for key events can be used if shape the form of the dose response to the concern. Allows the ability to calculate the possible threshold for something called the transition dose value and the quantitative dose by the means to determine whether this linear or nonlinear speculations. Which one is appropriate. With additional support for the biological perspective and of which model is an appropriate model.

And as far as Risk21 framework we believe this is a problem formulation-based. All about exposure. That's one you want to consider between doing any toxicity gathering of data. [Indiscernible] to be constantly fed back in. Matrix to see how it has affected the risk assessment. And the modulate factors, systematic step fashion to address nonchemical [Indiscernible] provides very simple visual documentation for each process on the step. And this allows one to be resource-efficient. So the project now is in its fifth year and there have been requests now to have training on the risk assessment process. Service training sessions inducted in specific region in China and Taiwan, and one here at FDA. It's already starting to get a lot of traction and a lot of interest as an approach for conducting risk assessment. The next slide is my last one where I will point out a few our information you can contact HESI's Michelle Embray and she will be happy to provide you more information on this and the project and many of our publications. Thank you for allowing me to [Indiscernible].

I want to invite all the speakers to panel. And especially thank Rita and Craig as we move into this next section. So, I cannot actually see the chatbox again. So, I have a couple questions. I will wait to see what questions we get coming in first. Could somebody read the questions? Thank you.

11:45 am–12:50 pm Roundtable Discussion—Elaine Faustman, University of Washington, Seattle, WA, Moderator

Audience Question: The question is for Dr. Rowlands. Do you have any guidelines or suggestions on data quality for you Q-KEDRF, example, number of concentrations tested in the number of technical replicates and/or independent experiments performed to generate the data etc.

Rowlands: That's a really good question. And we actually have an example of the estrogen induced trophy. Taking that we would have no problem finding enough dose response data for rodents and ended up finding not much useful information really when we dug into it -- so we asked questions echoing to the data you think it's there and it's not there. And the reason that -- if it's an example in question is we need better dose response information and you need more of it. In order to do this kind of quantitative and set assessment. And it's really a call for having more dose response information in the dose response generated going forward. I think in the twenty first century the *in vitro* models help -- we hear we have 10 to 12 doses across the response dose curves and is very good the technical biological information around the uncertainty there. To generate very convincing dose response curves. So, we're dealing with historical data. But we are able to demonstrate it is still possible to use under something with [Indiscernible] action. And again, you're absolutely right. Question is the quality of the data is really going to be very important to determining how useful the response modeling for key events is really going to be.

Faustman: Great. I have a question for you Craig. Two examples [Indiscernible] as a moderator about these two examples were really very good. And in particular with the H Custer was struck by your list of agents [Indiscernible - low volume] [Indiscernible - muffled speaker] and also activate [Indiscernible]. Have you and your group made any discussion about how one considers this is background and in particular given what Lauren wants to talk about earlier today is sort of what is the state of the population before we start to think about this?

Rowlands: That is a great question. Because we actually have looked at the endogenous receptor activity in human blood published two years ago. And it is remarkable just how much background activity is in human blood that activates the interceptor. The cell model reporter to model for age receptor activity. That it was very, very high. And over 1000 times higher activity that can be explained by the background TEQ levels which for those of you don't study the field that is the heat equivalent activity. So, think of it just as TCE. But it was 1000 times higher activity in the background human blood then you would explain by the measure TEQ levels at blood and we kept scratching out head. How do we focus? How do we incorporate that into human risk assessment when you have a little [Indiscernible] activity in a sea of natural [Indiscernible] endogenous activity and some of that clearly was database because we also did a dive study in there and actually demonstrated if you have certain [Inaudible] you can increase it and stuff like that. Some of it is dietary but also something that is endogenous in the blood. And we don't know how to incorporate that. So, the question is what do we do under the circumstances how much additional risk if any would be adding if you have that much actual natural activity present. Do you have any ideas because we couldn’t solve it.

Zeise: And I think that this issue of what to do about background really is deserving of a lot more focus. And thinking through approaches to looking at the extent to which we are talking about in incremental increases in risk and how to maintain levels so that they are not significant so that whatever additions above what we have in endogenous and endogenous exposures remain low. And it really does call for thinking about the actual potency of the series of endogenous exposures that we have in comparison with basic activity coming in from the environment. And so, I do think it's deserving of a lot more intention than what is being given

now and I don't see a lot of activity in the area. So, I am just curious if anyone has seen any different groups trying to take that on.

Dourson: Let me just add a little bit because I have a little bit different perspective and a Lauren's broader question I think is good as well. It seems to me that experimentally you could go *in silico* or *in vitro* and or maybe *in vivo* and test the organism for its endogenous, in additional loads to see what the cell is doing. Remember now that the endogenous loads that are already impinging on the organism and its individual tissues and cells, the cell's responding to that. I mean they are. They have whatever enzymes they need to protect if that is indeed the right word, probably not. And they probably have done a little extra because of reasons that we all as biologists think are reasonable. So, if we could actually test that *in vivo* or *in vitro*, you could directly see what addition to an exogenous chemical might have on impact with endogenous chemicals. So, it seems to me that this test can be done. Now the theoretical expectation is what we have already talked about before. We have these endogenous chemicals and we have responses to them or situations where we know the body knows how to respond. We are anticipating on Mel Anderson's work or others, at least I am, that the cells overproduce these protective enzymes a little bit. I think it's a great question and maybe there's experimental work to Lauren's point, if someone is working on this I think it would be great to have maybe experimental support for theoretical or hypothetical supposition.

Rowlands: Given a list of modulating factors that can be impacting any one of these processes -- it is going to be difficult to find the right case. But I think what we observed was clearly a nice case study in the sense that you don't get any dioxins toxicity if you don't have a [Inaudible] receptor, so we know there's only one door in. And so, it gives you a nice target to focus on and how things can impact that one key event. Otherwise there may be redundant systems in any of AOP's and you can look at one aspect but then there's another AOP they share key event that's really you may miss that completely. It's really very complicated experimentally. I think it would be great. It's certainly the risk assessment makes for great thing to say something and better handle on. Right now, we're just qualitative, listing what we know can impact it in the question then becomes how to factor that in quantitatively and I don't know how to do that.

Faustman: This is Elaine and I would jump in a little bit here. Because I think there are two good examples where we do a bit of this work. Of course, I'm coming from the developmental area. So of course, when we start to hear [Inaudible] is part of one of the mode of actions we're always worried about at what stage and what tissues of how much differentiation occurs because it normal in many of our processes. So, we actually have undertaken a process for some of our *in vitro* models methodically characterize the background rate for change for many of these so-called response pathways across development. The second thing is I always want to make sure and sometimes I think we need to be just a bit humble on some of these pathways. We within the last five years have changed some of the levels of allowable acetaminophen along with alcohol because we think we're actually exceeding what we think are our ability to respond to that level of damage. So, I think I agree with everyone at quantitation of this is really important. I want to emphasize again background and normal activities of people take in to play. Now I'm trying to get my family to eat more kale and vegetables but it's a hard uphill battle and it seems be taken more alcohol rather than vegetables. So, we may be on the responses side here. But and I joke about this. I do really believe that as much emphasis is with on these pathways and depending on them for protective pathways which I do believe we are very lucky the way we've evolved but I do think we need to get some dose-response relationships for those just like we're doing for the others, the exogenous compounds so that's my two cents on that.

Audience Question: The next question is from Goshin Chen. It is directed towards Rita. What is current status of mutagenic AOP for cancer? Is it going to be published by OECD?

Schoeny: Okay, there's a couple of things going on. Yes. There is a group of us that already have put a sort of case study online through the OECD wiki for toxins b1 articulating a mutagenic AOP for [Inaudible] carcinoma. The whole AOP world is evolving very rapidly and a lot more rapidly than I think more than the time some of us can put in to it. [Inaudible] case study would like very much to come up with a standard chemical agnostic AOP for the mutagenicity leading to [Inaudible] carcinoma. It's probably not going to be available for the next round of OECD review. But we're working on various publications along those lines.

Faustman: Okay great. Obviously, there's a lot of interest in that -- that's for sure here. So are there any other questions on the chatline that we might want to go to?

Audience Question: This is from Dan Levy. Jim Weinberg has been making an argument about endogenous formaldehyde and other chemicals for a while. I am unaware of the group that includes the regulatory agencies which is trying to integrate this into risk assessment.

Schoeny: This is Rita. I'm reverting back to the love for my employee of thirty something years, I know EPA is aware of Jim's work, that absolutely beautiful elegant work that aims to differentiate between endogenously and exogenously formaldehyde in the extent to which the dose responses for added information. What is happening in the formaldehyde realm right now with the EPA, that I don't know but we are certainly aware of Jim's work.

Zeise: And just add to what Rita is saying I know that there was a workshop at the Society of Risk Analysis in December and EPA staff participated, and I think a variety of issues were discussed. I do know people are actively thinking about the extent to which these measurements can be taken in and there are a variety of issues in the timing of dose, the intensity of dose of the target tissue that is also being considered. So, what do we mean by background and measures compared to one might receive in a case where you have a very localized exposures yet another issue that is kind of trying to address the overall endogenous question in the context of the specific exposure to an environmental dose of formaldehyde.

Schoeny: And one thing that I have been dodging personally -- this is Rita -- is methylation of DNA as part of the epigenetic control and changes that take place in all the developmental systems. When you start looking at what's a mutation and what's epigenetic modification for signaling or development, I start to throw up my hands and I am hoping to [Inaudible] un-snarl some of this.

Faustman: And Rita I really love that you brought that up because I was living when my next areas. And I was watching sort of methodically when you talked about and Craig, your talk about epigenetics [Inaudible] in that slide number probably 18. Have you discussed this in the groups?

Rowlands: Actually, no. That's actually something I put in the paper because I know we have to have it in there. [laughter] because they can hit a lot of different places I agree.

Faustman: Also, I am struck by the discussions about the importance of time. Again, I am in development. So of course, we always believe the time is extremely important. This idea of time to tumor versus time at this stage even is age-specific or what the cells are doing at that time when the damage is initially occurring versus the time to win the tumor, there's a lot of activity

that occurs. So, I have always been a bit worried about the kinds of tumor is not being perhaps as granular as it needs to be especially now that we hear about some of the epigenetics language. And it also has its own dynamics on this and maybe more reversible and appliable than we first thought or at least in certain cell populations.

Rowlands: The challenge that we have we want to make this clear session AOP's which are really focusing on how to eventually develop assays against specific key events and then study those and determine how that fits in AOP toxicity. The temporal relationship for that exposure is really difficult to replicate. And yet we go back in the receptor – plenty of the age receptors that are not toxic. But you wouldn't know the difference in an *in vitro* assay which is exactly the same. So, from a developmental perspective, we certainly have better experience in the timing of toxic and how they can impact toxicities in developmental sects. But in adult animals we don't do that for whatever reason. There may be a genetic component of that that maybe that would become another marker, another thing that we can add to the test. But right now all we can say is that we have this activated for a very long period of time before we eventually produce a critical next key event that [Indiscernible] I don't how to factor that in yet *in vitro* yet but we need to think about that.

Faustman: I was just saying we use to manipulate that a lot in cancer progression by adding instruments to partial [Iinaudible] artificially elevate or modified that [Indiscernible] responses. So yes, there are some techniques there that we haven't related the back to reality.

Zeise: Yes, I guess just thinking about Craig's last comment. It also raises the issues of sort of the propensity of the binding and the extent to which it might persist. And genetics is a very important piece of that in that understanding that in some detail might get you at different weightings of different kinds of exposures.

Rowlands: Kinetic factors definitely factors into this class of chemicals in their toxicity. But [Indiscernible] would be very helpful to factor into it and perhaps other classes as well.

Audience Question: Okay I will interject at this point. There was another question I came in online that is dealing with the time toxicity of your speaking about right now. The question comes from Janice Bilal. The question is you think that risk assessment 21 is overlooking the dimension of time? One can think of the key event as being a rate-limited enzyme reaction such as DNA repair. To precisely measure a rate limited event, we need more time precision. WDYT. I suggest that we begin to capture the time dimension approaching this scale/precision by applying the new real-time imaging technology. What do you think? Can risk assessment be improved by adding more precise measurements of those rates?

Rowlands: Agree totally but we sought to understand the timing [Indiscernible]. Right so [Indiscernible]. That physiological comparison is going to be important to understand when you measure time dimensions it could be good in an animal model versus human depending on what you're looking at. But in general, more information over time is definitely better.

Dourson: This is Mike Dourson. I was just going to say that the idead of incorporating time depends on part on your problem formulation for the cancer endpoint. It really is a lifetime of progressions. So, the disease is usually a lifetime progression. I like the idea of the in-utero exposures and we have different kinds of events going on at that point that sometimes weigh in on the lifetime cancer event. So I guess it is a lament and really an agreement with Craig and the questioner if they did have this kind of timed events, we could get a clearer picture perhaps of the underlying mode of action or mode of action or mechanism of toxicity and if we had that

clearer picture, it would somehow make a difference on how we assess the risk. But then again it goes back to your problem formulation because that has a difference on how you look at time events.

Faustman: But Mike I might argue back that for a long time we have had fields that short-term limits exposure [Indiscernible] that recognized in the occupational setting. And part of our arguments about formaldehyde or the fact that we aren't using scales and thinking about short-term very high doses that are exceeding repair capacity at these windows. They are actually happening in more places. So, it seems to me that maybe we need to look back a little bit more about what we know and where [Indiscernible] has not been such great idea. So maybe that was too simplistic of ways to think about stuff. Well of course it was. But I don't think we should take carcinogens off the list for needing to look at time aspects of this.

Dourson: Elaine I agree with you completely. We do handle time aspects of carcinogens in a very crude way [Indiscernible] relationship in 1967 with the dose rate times latent periods raised to a power and the power averages three. That's what people use in their adjustment, that threefold multiple of the slope when you are getting tumors much earlier in the two-year bio assay or whatever. It's a crude adjustment. It is in the right direction. Can we do better? Oh my gosh I hope so.

Faustman: I would just think that the dose time concordance table really offer some opportunities to look at that bit more.

Dourson: Oh wow, they certainly are. I am so glad that Craig showed those and that Elains brought it up as well because when we started doing that for our own assessment, it was eye-opening. I mean modes of action that we thought were well-established were not. And modes of action that we thought well you know that's not such good mode of action for whatever reason we found the data lined up even though the data was not powerful on each individual key event or maybe modulating factor. When you added them together it showed the story. So yeah, these time dose tables are really important. I encourage everybody to do that.

Audience Question: This is my question that we were discussing. And my point is that it's the dose rate that makes the poison and that we now have the tools to allow us to get to dose rate. And I would like to see us major in dose in the dose response curve as the dose rate response curve. And maybe even rate response curve and I suggest we can do that by applying the new technologies real-time [Indiscernible] and real-time chemical exposure assessment.

Zeise: This is a very old problem and well-recognized problem and in my seeking being able to explore that further in animal studies is very exciting. With the tools and when you think about all those occupational dose response curves where the cumulative exposure measures are saturating to the extent which that might be due to dose rate effect.

Audience: I would like to suggest that the threshold limit may be due to difference in dose rate and if we were measure as dose rate maybe that threshold would go away.

Dourson: Janice, this is Mike Dourson and I have a question for you. The way we do risk assessment in regulatory science is dose rate. Again, very crude. And the recent I think the assessor do it in a crude way is when we try to link that to our regulatory colleagues, they talk in terms of use of 2 liters of water a day, which is no one unless you are a teenage boy in football practice, who drinks two liters of water right now. Well, some people do that, but the point is, it is

a crude measure of dose rate. And if we can improve that crude measure with maybe ideas that you are suggesting that would be very helpful.

Audience: Mike my response to that would be in the adverse outcome pathways. We're really looking for the rate limited steps. And we need to have the time measurement approaching the component.

Dourson: Agreed. We are going from something that is very easy or simple superficially -- how much water do people drink per day. 2 L of water per day and then to what's the concentration of water to what exposure does to internal dose. And we do something simple to back that up – the reference dose is some [Inaudible] per kilogram per day -- so all of these are very simple things. If we can get a step towards dose rates for AOP, I think that would help as you are suggesting, tremendously.

Audience: I think in the future we will not see a dose response curve, we will see a dose response topography that changes with time.

Dourson: Okay. Thank you.

Faustman: And one could imagine that happening in workplaces. Jan, I am glad you jumped in because when I heard my name, I was wondering if the question was yours, but it became hard to tell from the new pronunciation of your name.

Audience Question: Okay this question is from John Bowers. Mike if you could elaborate why problematic to identify thresholds statistically? Is it lack of sufficient data over range of doses? We have seen a couple examples in today's presentations where there seems to be sufficient data to see a classic pattern. Still insufficient to this case is. Wondering what the Crump paper is you are referring to John Bowers FDA.

Dourson: So, let's go back to the basic idea. It's mathematics. In this particular case or tool that us biologists, or toxicologists are using to understand the data. The data you know is simplicity. The data rule, the data are correct. The tool is trying to understand it. Now what McKinney Crump has done, and he's done it very well and he is basically how I will paraphrase [Indiscernible] correct me. Is that [Indiscernible] cannot make the distinction between the threshold or not. He can give you mathematical model that looks like a hockey stick, but there is no threshold and etc. [Indiscernible] had no threshold but it was a hockey stick. Or you can do different data model that you would suppose is no threshold and it is linear, but it is. So, what you have to do is you have to understand the underlying mode of action. And more specifically the underlying biology and what people have simplistically done at FDA is was the lead here back in the 50s, there's going to say hey, we will think that mutation causes tumors and it could be one chemical so therefore the threshold is one chemical or one molecule -- that is literally what the word threshold means -- there is no zero doses without a fact and the other simplistic thing we said is you for chemicals like solvents you need to impact and kill cells and kill off the cells in the liver even notices it lost a cell. And that's a threshold for the adverse effect. We have a lot of people argue while there is no threshold. And when you really talk to them about that, it is because if you put one molecule or chemical in your body, it will cause and effect somewhere. So, if you take a water molecule and put in inside your mouth, it will cause and effect if you measure it. Is that above threshold? The answer to the analyst is it's not an adverse effect. You have always very simple concepts that work well for regulatory science. Now when you step into getting into this getting into the exquisite biology in which we are now in, and this is a good thing, it's going to take many people to not many, certainly more than one, to look at this kind of

information. To understand the biology well enough to see if the cell is to the point where you can have a threshold for adverse effect or not. When people say you can't justify or you can't prove thresholds, that is not correct. You can prove thresholds all over the place biologically. Otherwise, we wouldn't speak. And goes on and on. But that's not really the question. The question is for this particular event, this is what we are calling an adverse effect. To have a threshold for that effect and now we can say what is the mode of, what are the key events before that and to be have a threshold for the key events. And that understanding biology is really where we are going to end up. And that's why one of my conclusions was, we need multiple people in this. We have to work together on this. Because not any one person understands the nuances of cell regulation. I certainly don't. And you need pathologists to be able to tell you what they see happening. And how it fits to the up regulation or not. So, I think we are all in this together and I think idea threshold. It's still an important question I think from a regulatory science point of view. But from an understanding biology point of view, that is really where we will end up. So, I hope that answerer your question. Hopefully there was some light shed there.

Zeise: I wonder if I could add a little to Mike's explanation and kind of take us back to know the beginning with the basic assumption of linearity for carcinogenesis. And I think you know it all relates to background processes. So even if there is a threshold at the one or two molecule level, even for a chemical agent -- to even pick dioxin -- we are so far from [Indiscernible] all very big numbers and we are very pretty far from having just one molecule of anything. And on top of that, we have biological processes. That are operating in the exposed population and at the same time as the disease associated with it also occurring at a reasonably high-level. So, this issue of background directly relates to thinking about thresholds. And I think one of the challenges is to see how we can begin to consider some of the basic biological experiments which are done sort of in the absence of a variety of exposures. And health status dates and see the extent to which additional exposures that humans might be experiencing and alternative alterations how we might begin to consider the *in vitro* and *in vivo* studies in light of these other background exposures.

Faustman: I wanted to go back a little bit. I mean we've had some discussions about when do we have sufficiency of evidence to move forward or not on these modes of action. Do we want to come back and talk a little bit about that because I think that's a generic issue that many people actually brought forth. Any takers on that one?

Dourson: Yes, this is Mike and I can weigh in. But I tend to just jump in. So, I will maybe be quiet a little bit if somebody else wants to jump in first.

Rowlands: Mike, I will go ahead and jump in. I think the I mean the short answer is it really does depend on the regulatory question you ask for problem formulation. So if we're going to prioritize chemicals, we may be using a key event from the mode of action -- we use for the endocrine screening program. We use endocrine pathways and receptor activations for hormones and there is plenty of uncertainty allowed in that. We're just using a single key event but we're using it in a way that sorts chemicals and in to rank order for those that are testing or further trusting or another not using conventional approaches. So, I mean there is a mode of action based scenario where testing a lot of uncertainty there not a lot of data necessarily. That whereas if we want to use this for read-across maybe we deliberate more information understanding mode of action. But we least it extends target tissue may be -- something like that. But if we're going to use it for hazard prediction, then we really need to understand this is where the AOPs are going to have to come in, really understand pretty darn well what it takes to produce that hazard on an *in vitro* scale. And that's going to require a lot more information

obviously to do that and more research. I think one of the areas that we can use mode of action information to actually do risk assessment even today if we wanted to be not worried about predicting hazards anymore. But just identifying safe levels of exposure to humans based on the biological activity of relevant end points the relevant biological activities. And here if we can have some confidence that we are screening biological space, so to speak, that is representing majority or whatever percent we need to cover I don't know 95% of all the known biological pathways that toxins could affect, is that sufficient to just use this for margin of exposure and not worry about predicting whether a chemical has some particular toxicity because all we really do in theory is find out where that doesn't occur anyway. And use that number to the determine the margin of exposure that's safe anyway. So, I think there's different ways to look at it and going forward we need to step back and reassess what we're trying to do here in toxicology risk assessment. Predictability or to find safe levels of exposure? And I think that is going to determine how much information you need to go forward.

Schoeny: My guess is that we will all jump in -- [Indiscernible - low volume] the process formulation is what determines how far one goes in terms of any kind of demonstration that you make. The other side of that question is one reason it has been so difficult to come to closure on a framework or mutagenic mode of action…there were objections to their number of reporters that the burden of proof was quite high and in fact, it is. But I think that's the case for a good demonstration of any sort of mode of action.

Faustman: I will step in. This is Elaine. I think it was interesting Craig. Two points, that you choose the EDC endocrine receptive pathways for example. I think it's not only about making new decisions about ultimate actions. But the recent Federal Register notice saying that maybe what we have enough information now that certain assays are more predictive or as sufficiently predictive with other assays. And suggesting in downplaying the Hershberger assay. I think we haven't had that type of conversation here yet, but I think that by having more information having a mode of action and what we're looking at will help us in these predictive assays also. That was one thing I was going to say. So, I was pleased to see that use that as an example. In the second thing then is someone made the reference to thousands of different modes of action. There's new papers coming out and many others talking about maybe 10 or so mechanistic pathways that we should be thinking about what we use the with the modes of action. But I would think a lot of implications from what we are hearing this morning for how we go about and moving forward. And I think it's actually getting some of the generalities are becoming more interesting.

Rowlands: I was is going to -- and it really does depend on whether you actually want to predict cancer or just find a safe level of exposure.

Zeise: So I do think just on the question, if -- you do see from a regulatory point of view, different actions if it looks like the effect is going to affect the potential of the young or developing fetus and the nature of those effects, not just that it might be kind of -- we have biological activity and we can order means that within you could be something that is adverse versus, oh, this could impair basically the development of this child. I think it might've been Rita that mentioned that it all depends on risk management framing. And this group outcomes in terms of basic hazard traits with an understanding of the severity of his hazards I think makes them more helpful applications.

Dourson: Mike Dourson here. It's a nice question, Elaine and I think we've all hit the major aspects of it. Formulate your problem first and if you don't really care so much about --because you have to make a decision -- the excess lifetime cancer risk call. Which we all know is likely to be conservative. If you can still regulate the chemical or whatever it is on the basis of that you

can be done with it, that is fine. If you have exposure that is less than whatever works out to be -- and 10 mg a day which is extraordinarily low number that is a milligram kilogram per day equivalent to the threshold with pathological concerns for the most sensitive thing which is the genotoxic carcinogen, well then, you're done. You've formulated the problem where no one will worry about that exposure is not because it's so important, but from a regulatory point of view the SBA does not -- the law doesn't deal with trifles. That's kind of the regulatory aspect to it or it's below the threshold. Either way, it doesn't matter. In situations where your exposures were different and you take acrylamide in food -- is one of the examples we did -- 40% of the food has chromite so kind of working out the level that is associated with the cancer risk with uncertainties is important. And then looking at mode of action in more definite intensity makes a difference. We did this, we did a dual mode of action based on EPA's guidelines and I know dual mode of action is not the current [Indiscernible] but it was the mode of action that had two aspects to it, direct mutation and a simulation afterwards. And we did the analysis and dose respond that way, it made a difference for certain types of food being problematic or not -- whether they are problematic and I don't know -- but the point is the problem formulation was different in that particular case and we had my data, we come to the situation that was with different kinds of risk management outlet. And along the way it is not any one person or group that gets to say this is how it is. And we to work with all of us colleagues together because we operate in different piece of this. This assessment puzzle and I really think we should be doing this collectively. That's where our best decisions are going to end up being made.

Faustman: Well that's quite a bit of collective activity as you mentioned yourself that so let's check that chat board again and see if there is anyone else as posing questions to us.

We have no more questions in the chat box.

Faustman: So we have about five more minutes than we can go around. Are there any more subjects at the panelist wanted to bring up?

Zeise: So, in terms of something for Rita in terms of and the multiple modes of action. And as for carcinogens and very early on -- I think it was maybe Sam Cowen. Saccharin is not much of a concern for human consumption but it was a very interesting case where you gave the compound in-utero. And in-utero exposures and had a rather high bladder cancer incident. And was proliferative. I think the effect is proliferative and targeted and dose rate effect. And similarly, for DES that also has the mixed exposures. DES is of course both mutagenic and does cause proliferations of hormones. And so you have these mixed modes of action and you have exposure very early in life that really sets up process that really sets up a process that you see manifestations of many, many years after and I am wondering about your thinking with respect to these early life exposures and the factors that were developed in terms of what could be called jackpot effects where you have some key proliferation and you just as a very early in life.

Schoeny: I don't know the in-utero studies on saccharine really at all. So quite frankly I have been going through my adult life happily counting down all the saccharine I can but that is a different kind of exposure. One reason why I have been so enamored of adverse outcome pathways is that they are kind of short linear not in the dose response sense but in the progression sense. Pathways particularly and then I think link and interlink depending on the type of exposure that one is trying to model. So again, I think that these are going to help us deal with the number of different kinds of susceptibilities by once again taking a problem and chopping it up into smaller pieces and then reassembling it in a reasonable fashion to get at some of the complexities that we know is out there without getting completely lost in the details.

So that's kind of a philosophical approach to approach that you ask. When the interim guidance came out on the supplemental guidance, it was based on very few data. I think, and you and I were both I think in SOT meeting where we had a discussion of five years after -- how far have we got in terms of trying to understand early life susceptibility for various kinds of chemicals in the processes that they induce might not be a bad time to take another look in the light of more understanding of processes by virtue of breaking them up in some of the smaller steps. The old ABS were they sound very, very few data as we know. Very few studies. Not a bad idea to take another look right now.

Faustman: Well I think we will end on that note. And Rita I think that the NIEHS is hearing you bring you the national toxicology program because of their commitment to start in-utero carcinogenesis bioassays. So, your comments have not fallen on deaf ears hear. So, I want to step in here because I want to thank the SOT and FDA for providing this virtual space. Actually. virtual because of all the snowstorms. We want to make sure that we think the organizing teams and I know Suzy Fitzpatrick wanted to join numerous times and I will send email back and forth so I don't know if she actually got on -- did you want to say anything about do you think we met the goal that you set out for us? I am taking that resounding silence as a round of applause here. [laughter] So, Suzy thank you so much.

Fitzpatrick: Can you hear me Elaine?

Faustman: Oh yes.

Fitzpatrick: Yes, I think you met your goals. I think everybody did a super job. This has really been great. Especially under the circumstances and I think we are taking a lot of things to think about from this workshop. So. thank you everybody. And especially thank you to the AV people who are top-notch -- we are so lucky to have you.

Faustman: And to Betty. She made it work. Again, round of applause for everyone here.

[Event concluded]