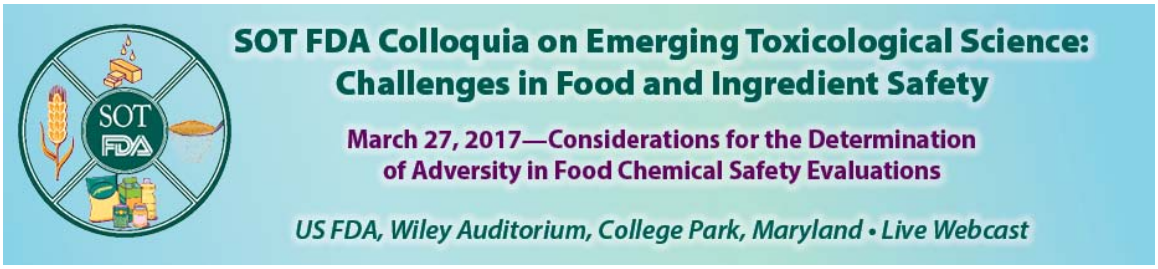

Considerations for the Determination of Adversity in Food Chemical Safety Evaluations

Chair: Bernadene Magnuson, PhD, ATS, Health Science Consultants, Inc., Mississauga, ON, Canada
Co-chair: Sabine Francke, DVM, PhD, Fellow IATP, CFSAN, US FDA, College Park, MD

8:30 AM–8:35 AM	**US FDA Welcome and Overview** **Suzanne Fitzpatrick**, PhD, DABT, CFSAN, US FDA, College Park, MD
8:35 AM–8:50 AM	**Welcome from SOT and Introductions** **Peter L. Goering**, PhD, SOT Past President, US FDA, Silver Spring, MD **Speaker Introductions: Bernadene Magnuson**, Chair, PhD, ATS, Health Science Consultants, Inc., Mississauga, ON, Canada; **Sabine Francke**, Co-chair, DVM, PhD, Fellow IATP, CFSAN, US FDA, College Park, MD
8:50 AM–9:30 AM	**Adversity in Regulatory Science: Historical Perspective and Future Challenges** **Nigel J. Walker**, PhD, DABT, Deputy Division Director for Research, Division, National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC
9:30 AM–10:10 AM	**When is Adversity Legally Cognizable?** **Ricardo Carvajal**, JD., MS, Hyman, Phelps & McNamara, P.C. Washington, DC
10:10 AM–10:30 AM	Break
10:30 AM–11:10 AM	**No Observed Adverse Effect Level: Sucralose as a Case Study** **Bernadene Magnuson**, PhD, ATS, Health Science Consultants, Inc., Mississauga, ON, Canada
11:10 AM–11:50 AM	**New Approaches to Adversity Assessment in Food Safety Evaluation** **Daniel Krewski**, PhD, MHA, NSERC Chair in Risk Science, Professor and Director, McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Ottawa, ON, Canada
11:50 AM–12:50 PM	**Roundtable Discussion** Moderator, **Bernadene Magnuson,** PhD, ATS

Reference recording via streaming link https://vimeo.com/211657870 or from SOT website
The text below is the captioning of the live colloquium, not an official transcript.

8:30 AM–8:35 AM US FDA Welcome and Overview
Suzanne Fitzpatrick, PhD, DABT, CFSAN, US FDA, College Park, MD

Renée Madden
My name is Renée Madden and I would like to welcome everybody here to the Wiley Center. I just have a few things that like to say. For those that are drinking coffee please when you finish take your cup with you and please try not to spill anything. If you need to go to the restroom, you go out this door make a left halfway down the hall the way you came in. In case we have an emergency, I need everyone to meet across the street at the Metro so that I will know who is here because we do have businesses here and we need to be accounted. Once I know that you have been here and accounted for, you can go to your respective location. Any questions? Please enjoy yourselves and Susan will give you the rest of the introductions.

Suzanne Fitzpatrick
Good morning I am Suzy Fitzpatrick and I work here at CFSAN and welcome all of you in the audience and online and we are here to talk about FDA colloquia on emerging toxicological science. We are talking about food chemical evaluations but the topic of adversity is an important topic for all toxicologists especially considering outcome pathways becoming so prominent. We have set up these colloquia a few years ago as a form to engage in new regulatory and non-regulatory toxicology to talk with stakeholders and the FDA Centers for Food Safety and Applied Nutrition and to engage leading toxicologists from around the world in this partnership. We have opened this to the public and we have had more participation from around the world for this. This is not a public forum to discuss with our agency the regulatory decisions that we make on emerging science, but it is intended to discuss high-quality toxicological science to advance regulatory science. So with that, welcome again everyone. I will now turn it over to Peter Goering, SOT Past President who has been helpful in getting this series going and we appreciate all of the help you have given us so thank you.

8:35 AM–8:50 AM Welcome from SOT and Introductions
Peter L. Goering, PhD, SOT Past President, US FDA, Silver Spring, MD

Welcome everyone it is a pleasure for me again to welcome you all here for this collaborative event continuing collaborative event between the Society of Toxicology and to be food and drug administration. I can think of no other collaboration that has been as successful as this one in recent times between the Society and another organization. We are engaged in efforts to provide state-of-the-art training for our staff not only here at the FDA but also at universities and companies and other agencies not only in the United States about around the world. We have about 65 or 70 persons in the room here, so a personal welcome to all of you. I also welcome the several hundred participants joining by WebEx from around the world. I believe, Suzy, this is our 11th colloquium. Ten are listed here. As you can see by the titles, we are offering state-of-the-art approaches to risk assessment and the regulatory science so I believe it is a very important and worthwhile learning opportunity for staff. I am pleased to announce that the Society of Toxicology and Food and Drug Administration renewed our memorandum of understanding last December. This memorandum of understanding facilitates the collaborative efforts that bring opportunities such as this to you. It allows for training, education, and professional development and the discussion of innovative toxicology methods and regulatory science. Here is a photograph with doctors Keith and Morris sitting in the front from SOT and Dr. Borio, the Chief Scientist at FDA and the number of representatives from SOT and the different at the a centers were also present.

I want to bring your attention to the next colloquium on May 23 of this year. The title is a Safety Assessment of Food Packaging and Food Content Substances. We have people chairing this session both from within and without the agency. This promises to be another good colloquium.

This event would not be possible without our outstanding organizing committee. Ivan Rusyn has been Chair of this Committee for the past two years. He is stepping away now and Brian Delaney from DuPont Pioneer will be taking over as committee chair. The committee is populated by members of the SOT outside of the FDA but also critically important, it includes members from the Food and Drug Administration.

It is with some degree of sadness that I report to you that Dr. John Doull died this past Friday. Dr. Doull was in his mid-90s and suffered from some poor health recently. As I said in his mid-90s we saw him at SOT meetings at least when he was at 90 moving around and engaging in conversations with many people. He had an outstanding career at the University of Chicago and the Kansas University of Medical Center. Many of you are familiar with Casarett & Doull's Toxicology. He was one of the two founding coeditors. He was the president of the Society of Toxicology and truly was one of our founders of the discipline that we know as toxicology in the modern area. He had a quote here he always shared at some of his speaking engagements, “Toxicology is what we do, risk assessment is why we do it.” I just wanted to invite you to share a brief moment of silence in memory of John. Thank you.

It is now a pleasure to introduce this morning's colloquium chairpersons burnout Magnuson and Sabine Francke from the Food and Drug Administration. I would like to introduce them in the order that they will introduce the speakers, so welcome to the FDA it is good to have you here.

8:35 AM–8:50 AM **Speaker Introductions: Bernadene Magnuson, Chair, PhD, ATS, Health Science Consultants, Inc., Mississauga, ON, Canada; Sabine Francke, Co-chair, DVM, PhD, Fellow IATP, CFSAN, US FDA, College Park, MD**

Bernadene Magnuson
Thank you very much, welcome everyone and everyone online, and thank you to the organizing committee for the suggestion, implementation, and this opportunity for me to participate. I invite my Co-chair, Sabine also to come up. I want to make one little comment; I just saw the readout on the bottom of the slide that translated my name as burnout Magnuson. I just want to say that this has not at all resulted in me being totally burnt out [Laughter] so just a small correction there.

Sabine Francke
I want to thank you all for coming. That is the number one thing. None of this makes any sense unless we have the people who come in person and online to listen to what we have to say in order for the benefits of the help that you enjoy it. We hope you enjoy what they have brought and we depend upon your questions for a very interactive panel discussion. We hope that you understand we will hold your questions until then so we can engage in dialogue. You can ask questions and other than that, we do hope you work with us and hold your questions for us to the end to give up the speakers there respect and we would like to make your session a success.

Bernadene Magnuson
So this will be a very brief introduction of the speakers. We will not read their entire CV that would take the entire section. So briefly, I would like to introduce our first speaker is Dr. Nigel Walker. Dr. Walker is the Deputy Director for this research of the Division of the National Toxicology Program at the National

Institute of Environmental Health Sciences of the National Institutes of Health. He has been there over 20 years and didn't disclose exactly how many over 20 years and during that time has worked on numerous and initiatives understanding the [Indiscernible]. In his current role, he is involved in the formulation coordination and implementation of activities necessary to carry out the goals of NTP and that involves developing tools in tech toxicology and molecular biology so we are delighted that you are here.

I will also introduce Ricardo Carvajal. He is the Director of Hyman, Phelps & McNamara, P.C., a law firm based in Washington DC and specializes in FDA and related regulatory matters. From 2000 and specializes in FDA and related regulatory matters. From 2002 until 2007, he served as an Associate Chief Counsel at FDA were he counseled on a variety of food related enforcement and rulemaking activities. In his current role, he is active working with a variety of clients on the regulatory status of the various ingredients, finished products, and providing guidance on compliance with new and emerging the regulations and being involved in disputes, welcome.

Sabine Francke
I have the pleasure of introducing Dr. Bernadene Magnuson and Dr. Daniel Krewski.
Dr. Magnuson of the Vice President Health Science Consultants, Inc. and has worked in the sciences of regulatory compliance for many years. She works with clients and challenges in the regulatory of novel food and beverages, as well as, dietary supplements. Dr. Magnuson has collaborated with many international scientific bodies in the capacity of promoting harmonization of food regulation, for example, with the US Department of Agriculture to promote education programs on food toxic toxicology. So we very much you thank for all of the work that went into putting this colloquium together.

Dr. Daniel Krewski is professor and Director at the McLaughlin Centre for Population Health Risk Assessment at the University Ottawa. He is involved in risk assessment with the new institutes of population health. He has served as an adjunct charge the faster of mathematics and physics prior to joining the faculty of medicine at the University of Ottawa. We are very happy to have him here; he brings a wealth of information and has kept us on the tip of our chairs in our discussions and in our operations.

We would very much hope that you can welcome all of our speakers with a nice round of applause this morning. Welcome.

[Applause]

Bernadene Magnuson
I think with that we are now ready to invite our first speaker, Dr. Walker.

8:50 AM–9:30 AM **Adversity in Regulatory Science: Historical Perspective and Future Challenges**
Nigel J. Walker, PhD, DABT, Deputy Division Director for Research, Division, National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC

Thank you. I just want to take the opportunity to thank the organizing the opportunity to come here today to talk about communicating basic science of toxicology it is one of my passions. So to give the opening talk here, this is a chance to get everyone up to speed on general talks principles related to

adversity, hazard, and risk so the subsequent speakers can then discuss and layer on more details. I would like to declare. I have no conflict of interest. My words of my own and they do not reflect any types of policies.

This is the outline of the talk I will give today. I will go through general principles of how adversity is interpreted in regulatory science and then we will move into some of the challenges for what I call "observed hazard." You will see what I mean by observed hazard in a moment. I'll then start talking about some of the new approaches to using adversity or sciences in decision-making.

So adversity comes in many different guises. To take this for example, the teeth are quite large. Clearly, the picture is a large predator. The exposure, I am pretty close, and it looks like a hazard and my risk is high, but there are three different things there. They are adversity, hazard, and risk. So how do we deal with that within food safety? We think about how we assess compounds that are in the food or food content materials, from what I call this adversity paradigm. This is where we have exposure, so this might be exposing cell systems or *in vivo* studies or controlled clinical trials, to certain compounds of varying dose that lead to some form of internal dose. This then leads to a molecular event that may be initiating events that leads to altered biological pathways, an altered structural function, and up potentially to death. What I am talking here is this continuum along the dose all the way to the ultimate adverse response. Bear in mind that this is predicated upon the timing or durations of exposure, individual susceptibility, and the environment under which the experimental unit may be. That could be co-morbidities or other health effects as well.

What we will talk about now is the definition of adverse. This is a consensus definition from a HESI Committee -- they change in morphology, physiology, growth, development, and reproduction or lifespan. It could be any change anyway along that continuum, the cell, or cell system that goes all the way to the population. That results of impairment of optional capacity or the ability to compensate. So it could be cell's susceptibility or an altered ability to respond to another, and in response those whereby they can't respond but will survive in a new environment. There is a slight discrepancy between adversity and activity and that will come into play later on as we talk about new approaches where a response may be an adaptive or adverse and we will discuss that more later.

This is a key thing to think about in terms of adverse responses. They can occur at all different biological levels from molecular response, to changes in oxidative stress, changes such as a necrosis, or neoplasia system-level things like description of endocrine signaling. Also, it includes individual level responses, such as changes in body weight, death, mortality, and all our population responses, such as lead and IQ distribution responses.

This goes back to fundamental toxicology. All things are poison, and nothing is without poison. Only the dose makes a thing not be poison. If we were to rephrase that in the regulatory context, what we are saying you're is all agents are adverse. The challenge is to what degree and at what dose. That is our regulatory challenge in the toxicology to understand the context of hazards when adverse responses lead to something being classified as a hazard and ultimately situations where you may have risk.

So now, we will move into the second part of the talk where I will talk about adversity and regulatory science. So let's go back to our opening slide, so this is a highly adverse situation the exposure is high, and my risk is high. This is an important concept in that it is the hazard integrated with exposure that leads to risk. All too often, we focus only on something being a hazard and forget about its context. In this new context, we have a slightly different situation. We have the same hazard, but we have

mitigated the exposure, so the risk as a whole is a lot less. In other situations we have, hazards may not be the same, and so we essentially have no hazards whatsoever even though we have a high level of exposure and this is an important concept for us to understand as we go through this morning session.

This is the integration of exposure, and hazards, and dose response for risk assessment. We think of the classic path to risk assessment paradigm we look to identify hazards based on adverse responses characterized with a dose response integrate with human exposure which can lead to risk characterization. So again, we have mentioned adverse responses can occur at a variety of different organizational levels all the way from cellular world -- to individuals. Also, timing of exposure and duration of exposure is important. Some responses require short windows of exposure and some require exposures during critical windows. Some require chronic exposure and some sub chronic exposure. Also, it's a hazard to whom, an individual or a population? Some are more susceptible than others are. So this is where current toxicology testing comes into it because current talk designs are based upon the identification of a hazard particularly the identification of adverse responses that specific doses that can be used to identify subsequent decision-making.

So in terms of dose response with assessment, we are often trying to identify critical responses and these are significant. We can use those to identify adverse effects level and or we can use it to do some benchmark dose response modeling to identify a level of response and different agencies use a different approaches but this is the fundamental concept. Therefore, this is a classic toxicology with increasing dose. We have an increasing dose response and these correspond to the dose groups used in a study, it could be a clinical trial, a specific toxicology test, or an *in vitro* test and we can identify the effect level and possibly a benchmark response leading to the identification of a specific point of departure.

These are the known outcomes that come from dose response analysis LOAEL, NOAEL, and benchmark responses or lower bands, which are the lower points, identified on the scale. [Indiscernible] The NOAEL must be an experimental dose. If you think about that, you can it and doesn't use all of the information of the dose response curve. Sample size can also affect the NOAEL as it goes further down the dose response you have less power to actually identify that critical effect. Also it doesn't actually identify dose responses at the NOAEL, it will again a vary depending upon the design.

Again, the benchmark dose method is where we used the whole dose response to identify a response that is corresponding to either some increase in background or a statistical response relative to the maximum. This uses all responses but it produces of the same thing a dose at which you are comfortable of either something not happening or a low response effect. This band would feed ultimately into the integration of exposure assessment that leads to risk characterization.

If we go back and have a multiple endpoints feeding down into the identification of the dose response, it could be at a cellular level all the way to a whole animal level. So how do you deal with different types of data because you'll have different adverse effects and adverse responses that could be from animals or human studies or from experimental *in vitro* studies and when we come to the evaluation of hazards the whole characterization of hazards we have to integrate these [Indiscernible] data. They come from different models or Cell models that come from different levels of data trust. We do GLP studies, which have a lot of confidence, and we know what was done. In other situations there may be more or less clarity about what is actually been done in the study because the guidelines are not there but ultimately the integration of this data is the critical step in the identification. This is now distinguishing adverse responses from integrating adverse responses into the classification of hazard that is then used to identify levels of risk. We're using systematic review approaches where we look at different data

streams to assign confidence to different types of data so we can integrate appropriately to ultimately come out with the conclusion whether either a compound or a different type of exposure is actually a hazard.

To give an example, one of the mandates of the National Toxicology Program is to produce the report on carcinogens. This is for human hazard it is not a risk assessment it is the early step in classifying hazards so this can be based on toxicology but it also looks at the sufficiency of human data as well as data from animals. Mechanistic information can be used in the determination of hazards. That could be either to upgrade whether rodent data is more relevant or it can even be used to downgrade a certain approach or a certain type of cancer may not be relevant to humans and that will come into play and as we talk about it later on this morning. At different data streams, and we integrate those into the classification of hazard but adversity is built within the individual types of studies or data streams that are used.

I will give you a case study now not cancer but in general about the whole idea of integration of Biphenyl-A this is something that is of interest to the FDA and SOT. I have been working on this for a while because we have many ongoing studies. This is chemical used to make polycarbonate plastics, there is exposure from migration from very small amounts in food, and there is a considerable debate as to what risk we determine a low-level exposure. Guideline compliance is studies have showed no critical effects at low doses and that has been the basis for the FDA registry decision-making and there have been many academic investigative studies that show a variety of adverse effects and a variety of systems at low exposures. This is an area of intense scrutiny at the moment.

One of the things NIEHS and FDA are doing is a consortium linking what we call academic and regulatory insight into the toxicity of subject, and how to integrate data streams from different types of experimental models and different sides of the scientific communities. This is where grantees from this FDA and 13 academic grantees had the goal that the whole idea of increasing our understanding of the basic study design and trusting of the information that we have had a guideline compliant to pronatal study conducted under GLP where it has been a guideline. At the GLP, we know exposures and we can read and restructure to know a better exactly what happened from that. We actually provided samples and additional animals to the grantees who can then look at more investigative studies to layer on and there was a focus on looking at effects reported to BPA that wouldn't normally be as you said -- evaluated in a classic study.

Again, it is integration of both investigative and guideline approaches. It has been an interesting exercise because this doesn't often happen. Normally research is conducted in isolation from the regulatory decision-making. So this has been interesting because it had led us to increase communication during study design, we are trying to increase the confidence and exposure paradigm used for investigative endpoints through the use of GLP, and ultimately we layer this within our systematic review framework that leads to a reduction in what we call the risk of bias. Ultimately, the samples are all blinded and all of the data from both at the GLP work and investigative work was coded so people didn't know what necessarily the samples or exposures were. Then all of that data is deposited into a third party database and when this is all released you can actually make your own totally independent evaluation of the data not necessarily the same evaluation or that might be in the academic public occasions. This is again to reduce the risk of bias and increase confidence in the decisions of so regulatory agencies can then make the best decisions based upon the best science.

So for the last part of this talk I will move on to some of the challenges or the observed hazard-based approaches. What I mean by that in observed hazard? We see neoplasia or critical changes but what

happens now if you cannot observe those responses? This is moving into what we are calling predicted approaches, the challenge is for many years NTP, and the regulatory community focused upon animal-based approaches, which in general have a lower response. The human relevance is much higher because we have been observed responses and we can have comfort to know how that translates into human hazards and human risk. There is a move towards a more mechanistic approach, which allows us to make assessments of chemicals in a much higher throughput. But the distance from the ultimate reason why we are doing it getting back to the approach that Peter will discuss risk assessment is ultimately why we do this not to have some -- how do we bridge this and gain confidence?

This is caught many of you are familiar with Toxicology in the 21st Century this came out in the whole series of reports in the mid-2000 the NDP for the 21st century and the NIF Toxicology in the 21st Century report. This spurred a lot of interest and a lot of work and a lot of action in the toxicology communities. The concept here is really thorough early evaluation with its altered biological changes and pathways looking at those responses of those pathways and integrating with all human exposure and population-based studies. Calibrating the dose symmetry to come to some level indicator of the risk your again this is focused around biological prohibitions that are not necessarily atypical adverse responses that are more often seen.

The way we have been approached and we have Tox 21 collaboration between FDA and NIH camps. BPA and the approach of a capacity to look at these mechanistic projects we are looking at 10,000 different chemicals. The main goal is to identify mechanisms of action to cause substances to need further evaluation and to develop models so there's a recognition that we still have to understand what some of these mechanisms are. From these pathways and that, we will develop predictive but we can also use them in the interim to prioritize more in depth about animal testing so there is a bridging strategy.

Again, this is all predicated on the live predicted adversities so there is an assumption here that a change in a biological pathway can be used to assess the possible probability of adverse hazard. We are no longer actually identifying a hazard we are in identifying an increased probability of 100 and that is a fundamental difference from where we were in the past.
For going back to our adversity paradigm, we have exposure, dose, and ultimately some type of hazard or adverse response. This is where the focus is now on these earlier molecular events and earlier altered pathways. Again, you need to layer on the impact of the environment, individual susceptibility, time and duration of exposure, and dose response. It is just that we have shifted the onus from being from this end of the continuum down to this end of the continuum.

So what are some of the challenges of using some of these predicted approaches? If you think about the initial perturbation predictors, the adaptation can reverse but such changes but so we know early biological pathways can go up and come down. So now the distinction between what is and adverse response, an active responses more murky, and we need to accept that and update science to do what to determine what the system is and sometimes this is not necessarily, what we would classically considered to be adverse. Agents may present in multiple interconnected pathways and so we may actually look upon no longer just a single linear pathway but more what I would consider to be more of a system disturbance. In at way, I think of that as an analogy of traffic and there is a wreck on the side of the highway. That may have shut down one road but what happens everyone spilled out into the surrounding areas of all of the traffic in the neighborhood can be disrupted and yet there is no wreck and that's what I mean by system disturbance and we need to think about some of these pilot -- biologic pathways with that in mind. Should we categorize these? We have to put these into the context of what is normal.

Many biological pathways can be activated by normal stresses that we are comfortable with so the [Indiscernible] normal and how these biological pathways or systems change relative to that normal states will be a critical to determine what is adaptive versus what is truly outside of normal that we might even consider to be adverse. The analogy I give you is if you only studied hurricanes, you would think that wind and rain was the adverse response because that's all you see. So the analogy is if you only study atypical observed responses *in vivo* this would be the same situation but we know that depending upon the duration and intensity we can deal with that so think how to use the weather analogy to show you that we need to fully understand the continuum of the exposure.

So in the last seven minutes I will talk about new approaches to adversity. There is a classical adverse responses and new predictive approaches and the question is do we always have to be buying adversity whether predicted or observed? Or are there other options?

What I would like to call is Sentinel responses. This is using a bioactivity as early low-dose Sentinel indicates that -- indicators of responses but in this case, there is no assumption that those early indicators are leading directly to an adverse response. That sounds counterintuitive that I will explain. This might be biological measures or gene expression or altered a transcriptional pathway that if something is happening in a coalmine this it gives an advance warning that a lower dose of something that may happen at a higher dose. So we are moving their paradigm now to a dose based decision as opposed to adverse response or predict it adverse response position.

Going back to the dose response analysis, take doses are coming down and we have NOAEL and our response, so if you have measures then maybe you can use some measure of the BMD of or the dose to be an indicator. If we use this as the point to go up from then maybe we can make sure that we are making safe choices or decisions about exposures in the absence of know what happened at the higher doses significantly. The extrapolated down as we have done traditionally in the past.

This was a started years back [Indeterminable] talks science did a study along these lines that looked at multiple chemicals and additional responses in different organs. Different routes of exposure and he was analyzing the change in gene transcription pathways in using benchmark dose to look at the low dose where those pathways have changed and compare that with the benchmark response from the atypical adverse responses that we traditionally see. You can see there is a reasonable correlation and this is only 10 chemicals and a few responses but it is a pointed in a direction that maybe we can use this correlation to use the benchmark responses from the biological pathways even in the absence of the truly knowing what those pathways mean to make the decisions.

People have taken some of this end, and done even more elegant comparisons looking at traditional approaches where they are looking at points of a departure for multi-organs identifying exposure and again the point is that the comparable points of departure regardless of the approach so maybe we don't always have to define in the adverse response. Maybe in some situations part of the screening program we can use the dose based approach and depending upon how the modern -- model of exposure we can maybe then layer on more science and tailor the approach depending upon the complexity of the exposure, and what the needs are of the data group.

The benefits are that this may be a low dose indicator of system disturbance it may give an early estimation of a point of departure of decision-making and diagnostic. If asked and we have been criticized that the exposure or the responses of that occur at high exposures are irrelevant when you go

down to the dose responses because of the wide extrapolation. Or maybe if we are actually making decisions of lower level of exposure, closer to normal been that criticism may be less unwarranted because you are actually looking at normal physiology is.

Similarly, we could potentially use human race cell systems to bridge the road to -- rodent to human extrapolation. Again, this is a real focus of what I am calling adversity to a dose-based decision-making. If you think about it everything we know about talks college he is the dose makes the poison and yet the way we are looking at adversity is the poison makes the dose and then we go backwards so maybe this is a way forward in certain circumstances to draw better interpretations for specific exposures.

Some of the pitfalls are at given points of departure may not lead to a hazards so people may not have trouble with that so if something happens and we don't know what it means it makes me the scientist very uncomfortable. The tissue that we were meant to be using maybe that may actually not be a good sentinel for a particular organ but may actually be the target of high concentrations. Of the hazard may be a different system so we may be too conservative in the POD or we are possibly not protective enough and this is the POD that may not wreck the sense of the other model. We have limited experience with this. This is a relatively new idea in the last 10 years and we have low comfort level in actually making decisions with this. What a two team strategies where we are actually moving forward with our traditional approaches but we bring along these approaches so we get comfort and those and experience with them and they are increasing our confidence to a certain approach to be useful and that's how we have to move forward.

So I am seeing yellow lights blinking so in summary, adversity. Observed or predicted is a central consideration and toxicology. I actually look back at Paracelsus and that was back in the 1400s or whatever so what would he say? Keep I would say this is me paraphrasing all things are adverse and nothing is without hazard only the exposure the dose the timing the biological context and the individuals susceptibility makes a thing have rest and I think that is a much richer definition in toxicology not just a simple dose.

With that, I will take some clarifying questions. We will leave in the bigger questions of higher issues for the panel but I would like to again thank the organizers for this opportunity and take any questions. Thank you.

[Applause]

[Indiscernible - speaker too distant from microphone] [Indiscernible - multiple speakers]

Audience Question 1
What did the NTP studies find out about low-dose effects?

Nigel J. Walker
It is actually still ongoing we are in the final phases of the peer review of the past college and we anticipate release later this year. We did a 90-day and a prelim to the two-year chronic study we did a 90-day study that was published two years ago and there were no dose or adverse responses in that perinatal ninety-day exposure.

Audience Question 1 Continued
Does that include the specialized studies?

Nigel J. Walker
No code that was only the 90 day study was a set of two dues to adequately designed the second phase clarity but there are papers that were written and people are seeing some responses are reported and posters on the effects of it. We need to see all of the data come together before we can make any high-level conclusions about that and the NT P output will be later on this year.

Bernadene Magnuson
Any other questions? We hope you will engage in the roundtable discussions. Thank you. So I wanted to thank Nigel for his excellent presentation and getting us started on a thought provoking discussion. I want to mention for those that are joining us online that if you have questions please communicate those through the chat function so that those will be well communicated and we will be able to read them. We also ask when you are submitting your question to also provide for us a bit of context as to where you are coming from and who you are representing so we are better able to address your questions. Please include that as you type in your questions into the chat. With that, we would like to welcome Ricardo and thank you.

9:30 AM–10:10 AM When is Adversity Legally Cognizable?
Ricardo Carvajal, JD., MS, Hyman, Phelps & McNamara, P.C. Washington, DC

Good morning everyone. I thank Dr. Magnuson in particular and this has been the best-organized efforts of its type that I have participated in. A quick run through the conflict of interest statement I am and attorney working in private practice. I develop substances for use in food, I will be talking about three case studies, I didn't have a diagram in any of those of slides, and beyond that, I have not received any compensation for this presentation.

So starting quickly with an overview, first there is foundational concepts and terms that are important since we have number of people not present in the may not be familiar with legal and regulatory systems. I will talk quickly about the history leading up to 1958, which is the landmark of food and drug in our country that gives us the framework that we have now. I will talk about that and then in 1958 Food Additives Amendment in detail that contains what we refer to as a general safety clause a general standard of safety. FDA has worked over the years to try to put some meat on that bone and get context to the word safe and the approval of substances in intended for the use in food so there is another definition of safe. Note hesitation could not be complete without Delaney clause and case studies to work with and close with key points.

This will be terribly basic to those of you here in the room but there are a number of people watching online may not be familiar with how our system is set up. We have a legislative branch that makes the laws, an executive branch that carries out the laws, and a judicial branch that evaluates the laws. All of these will come into play in this talk so FDA is an agency within the executive branch and is charged with enforcing the 1938 Federal Food Drug and Cosmetic Act and all of the changes that have made it to that law.

An additional background would come from two different cultures. I am a lawyer, you are scientists, and I am vastly outnumbered so just to make sure that since we have folks who are online and may not be familiar with our system they may use words differently than we do. So when we say a statute we are talking about a type of law in the food and Cosmo -- law that has been signed and a big condition on any law passed by Congress is that it has to be constitutional. The second term we want to have clarity

around is the concept of a regulation, which is a type of a law. It is legally binding and the binds about the regulator and the regulated industry, it has to be constitutional, and it has to be within the scope of the statute. So we cannot have a regulator running off to make regulations that are outside of the scope of the authority granted to that regulator by Congress.

Finally, that regulation has to be issue in accordance with procedural requirements and you have heard it many times but the basic mechanism to ensure fairness is common tools. Before a regulator can establish a legally binding, it must be noticed and there is a bit on a twist on that in the context of food additives, which we will talk. Finally, to it distinguish guidance from a regulation of a statute so a statute and a regulation about types of law in that they are legally going binding whereas guidance is not. The guidance is a thinking or interpretation about a regulation or a statutory provision that helps folks understand how the regulator is looking at those requirements.

I also should note I had of time that process doesn't matter so we can talk about safety and what that means and we can have a scientific discussion. There are procedural requirements to ensure that before all of those things can be folded into a context that places actual burdens on folks. There are certain procedural things that must be over, so the establishment of a regulation you have to. So if you have to go through hoops to put something into place after put down to unwind it and then also depending upon what pages of process you are into the type of process you are in the burden for who can shift.

Let's start with historical examples. Our national system of regulating food safety dates back over 100 years so we go back to the Pure Food and Drug Act of 1906 and already there were chemicals being used in food as preservatives. So there was already some concern about the potential impacts on health of those preservatives. We got our first national law you can see the standard in the second bullet and article food shall be deemed adulterated and therefore unlawful for distribution in commerce if it retains any added poisonous or deleterious ingredient, which may render such article injurious to health. There is a lot packed into those few words so one of the enterprises of a regulator is to unpack that and get meaning to each and every single word there but you can see some of the questions that that standard raises so when is a substance poisonous or deleterious? What makes it so and how do you put the balance around that? What constitutes an injury to health? Do we recognize any type of injury or is it only some type of injury? What does may render mean? So all of those questions are questions of that have to be answered by the regulator if they are going to put into place a regulation that I will explain what that means before they can go out to take a food out of commerce. Who bears the burden of proof under this particular framework?

It would be the regulator who was taking foods that were in commerce and testing to see if they put contained a particular poison and then decide if they would pursue an action against it. One of those actions actually gave rise to a lawsuit that went all the way to the Supreme Court and you may already be familiar so 1914 back then it was a relatively common practice to use nitrous oxide to bleach flour. So the use of nitrous oxide resulted in the technical presence of a certain types of cut substances that could be characterized over as a poison so this gives rise to what to do about that the flour that the government had received seized. What came out of this one is the mere presence is not enough if you go back to the standard right here it the standard isn't any added or poisonous or any. There is a whole bunch of words after that we have to make sense of the phrase, which may render such article injurious to health. Congress put that in there for a reason. So mere presence is not enough and fact it is has to be injurious to health. So injurious to the health of whom? Well it doesn't limit so we will conclude that injury to any consumer so this applies to everyone who is protected by that standard. Then may render injury is to health what does may mean well it will tell us but we have a dictionary so we have to go to a

dictionary of common uses and reasonable possibility and finally who has the burden? The way this is said, the government has the burden now to demonstrate that there is a poisonous or deleterious presence in sufficient quantities to give rise of reasonable possibility of injury of health, and that is ultimately what that arrived at. You can see that already we have various branches of government involved you have it the legislative branch that states that the standard we have the executive branch applying the law and we have the judicial branch interpreting what is happening to say “yes” or “no.”

I hate to speed through 20 years but in the time we have allotted this is about as good as we can do. In 1914 eventually, there was enough concern about the government's relatively weak authority to regulate substances added to food, which gives rise to the 1938 Act, which codifies Lexington Mills interpretation of adulteration states the standard and placed the burden upon the government to establish so the government can go out and demonstrate that there is a problem.

In parallel, you can see the developments of your wonderful science of toxicology and I just put these in because already think FDA has a background, which was put out in 1939. This is one year after Congress gives us the landmark legislation that discusses toxicity, chemicals, and food and subsequently updates to another reference by the national research Council as being relevant for purposes of a test. Detecting safety and in the meantime you have a growing concern among public about the safety of what seems to be an increasing number of substances being used in a production of food and you can look at the history of it does a congressional debates in the lead up to 1958. You can see a lot of back and forth to figure out if there was a problem and if so, what could be done about it by way of a national law and it is a very great legislative history.

We end up in 1958 with the Food Additives Amendment and this is a statute that is amended another existing statute for the Federal Food Drug and Cosmetic Act to revamp the approach to regulating the additional substances of food. So now we have a different standard so any substance the intended use is a key concept and to the intended use made reasonably be expected to have resulted directly or indirectly in a substance becoming a component of otherwise effecting the characteristics of any food, is the new standard in you can see it is an incredibly broad authority that has switched sweeping in any intentional use of a substance that can be reasonably expected to have an effect on food. It establishes a process for the approval of a food additive uses and the issuance of a regulation and there is a twist on that. I talked about rulemaking and we will talk more about how this process is different from that but in any case the idea is now there is going to be in substances food you can submit a petition. The burden is now shift to the proponent of the use of the substance in food instead of how to make the government happy to demonstrate safety and then we will have a type of her regulations which is a binding law put into place that governs the use of that substance in food.

We also get is a general safety clause, that talks about when the regulator can refute the petition. There can be no approval if a fair evaluation of data fails to establish that the proposed use is safe. The challenge is to figure out that word safe and I will talk about faith and also about fair in evaluation of data a bit more. Before I go on to that, I have to mention other important exceptions to the definition of food additives that comes up in our cases. That is the X option for uses of substances that are generally recognized as safe and how it is intended for use so you have general recognition science refuses than that use is accepted for that food additive approval process. In addition, because at that time we are ready had many substances on the market in 1958 a clear dividing point for some purposes so for things used in food before 1958 can be shown through scientific procedures or experience based on common use in food. In 1958 safety requirement for food additives is relevant.

The safety requirements are the same and there is some misunderstanding about that that this layers on a general element of safety is the same. I should also mention that we have a voluntary notification process for GRAS uses of substances in food so that is different from the mandatory premarket system for food.

Finally, what you have been waiting for, the word “safe.” What does a regulator do with that? Well the regulator looks to what or how it is intended, and looks at the statute itself. So the word safe refers to the safety or the health of man or animal so let's not forget that food is food and can be fed to both humans and animals and FDA has jurisdiction over both. A partition has to contain full reports of investigations made with respect to the safety of the proposed use so there is the idea again that because the petitioner has to detail exactly what it is they are asking and whether it is opposed to be safe. In addition, there is a number of things required above all consumption of the additive and any substance formed in or on food but cumulative effect on the diet taking into account typically or pharmacologically related substances and safety factors appropriate for the use of animal experimentation data. There was a long history of the use of animals in the experimentation for the purposes of determining safety.

So what does FDA do with those indicators of what Congress meant? In the first regulation, trying to implement this new authority issued in 1958. Shortly after, the FDA was deeply involved in the discussion with the congressional debates and essentially in a technical capacity as legislators thought about what to do they might go to the agency. Then so what effect does this have and FDA comes back to testify and provide feedback so FDA is ready to hit ground running and in 1958 proposed safe means that there is convincing evidence that no harm can come from the intended use of the food additive and that is a remarkably high standard. There are a number of things there that I will not go into in a lot of detail but I have it there and I will have this available for you afterwards so what are scientific procedures? That is the method by which we demonstrate safe safety. So what are scientific procedures mean? How does it affect tolerances? Of the use of safety factors? And with qualification better involved in these determinations and there is a point in 1957 a publication from NRC principles and procedures eventually is the touchstone that is the true source for determination of what they these criteria should be used and how any other approach gives equally reliable results.

We have a proposal than we have a final regulation that comes out after FDA has read through all of this and you can see this is materially different the first one was convincing evidence that no harm can come from the intended use and now look at this. Convincing evidence of which are established with a reasonable certainty that no harm will come from the intended use of food additives and those two words reasonable certainty are critically important in pulling up facts. From a standard like that perhaps no one can meet it. That one and now incorporates some element of reasonableness, which we can talk about in more detail, but you can see all of these reliable information to become unprejudiced reliable information both a verbal and unfavorable. So the idea that we want comprehensiveness of the information that is submitted and it is a bit of a change of the fall specific biological properties.

Fast-forward 12 years now, FDA has undertaken a review of a lot of substances that were thought to be generally recognized as safe and in that context FDA has to revisit is thinking about what does safe mean? So now let's go back into the legislative history of the food additives under the 1958 to pull out some of the thinking that would let us of the passing of the law to ascertain that any substance is absolutely safe, animal human testing is impossible and this is etc. It says that safe must be understood that to connote that FDA after reviewing all evidence concludes there is no significant risk of harm and you build out its thinking around this because there is this exception to the definition of food additives.

You have to think about the same safety standards and you have to start communicating clearly to folks what the standard actually means in practice if the law allows them to make their own best determination.

The other point is revocation of GRAS status that that's predicated on a responsible and substantial question of safeties. So have you reached the threshold of having something that requires legal action or legal standard than you have to think about to be unwinding and remember you have the first sets of safety that says general recognition that when are you going to revoke that GRAS is standard? Is it a potential issue or do you need to set the bar higher and the FDA is defining this is a substantial risk to safety.

So in the final role revises means FDA concludes that no significant risk of harm will result when the substances used as intended. A reasonable certainty language was taken out and we don't have a really clear explanation on the record or have not been able to find why it was taken out and FDA then signals its intent to issue a regulation prescribing the type of thoughtful logical data upon which the safety of the substance can be determined. This was a context of review of grant substances that the executive and the legislative branch are very interested in so there is a lot of interest in pressure building around these issues and initially FDA is thinking maybe we need to be clearer and more prescriptive in how safety is established.

Look at what happened a few years later, FDA has revisited the definition of safe and now we moved to do something that starts to pull all of the elements we are familiar with together a reasonable certainty in the minds of competent scientist that the substance is not harmful. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of any substance and in safety there are factors that should be considered probable both consumption cumulative effect safety factors and the benefit contributed by the substance. That is in an interesting thing the safety side will put all of these and now we have a new idea that not just focused on safety we are focused on benefits as well. That tells us when the proposal actually goes into final this is not now the modernist handed reasonable certainty not harmful under the conditions of intended use that is putting the final version to make it clear that we are always looking at this in the light of the intended use of the substance. The acknowledgment that we are not talking about absolute harmlessness and we have taken out the reference to benefits.

Along the way through this long history of the rule making, there are a couple of dates once that caught my eye because they are part of what we are dealing with. Regarding the scope of safety general safety clause applies to all types of health risk. Regarding interpretation of data, the methods and criteria for interpreting data are up to the discretion and expertise of the agency. So there is a mixed significant attempt here to assert flexibility and discretion. So much for the regulations, we talked about statutes and regulations. The Redbook is a form of guidance and I mentioned in 1939 version the first division's 1982 and we updates along the way and most recently we had a real Westpark comments on potential updates published in October 2014 and here a couple of key statements have been reinforced. What has been developed along the eight way FDA consistently has taken the position of various types of scientifically valid information may be used to support the determination that the proposed use of the determination is safe so we are not talking about a wooden safeties tended we have to put words of gratitude to help you understand. Appointed comes to types of data that might be used to support the safety of a given use of a substance that of necessity has to be flexible given the wide range of substances and uses we are talking about. Finally we have the statement flexibility and guidance for toxicity test needed. You have to FDA's guidance for toxicity studies, for food ingredients continue to

emphasize that there is no substitute for sound right to fix judgment that this guidance presents recommendations not hard and fast rules and not with the banning the statements. I say there is some misunderstanding of the Redbook and if somebody doesn't do this or that have they failed to meet the safety standards. These types of statements should be helpful to understand that better.

So setting aside the general safety clause, I mentioned the Delaney clause. So is a safety clause for comparison. Know of provable if a fair evaluation of the data fails to establish that the proposed uses. Then we have the Delaney clause of that is no additives shall be deemed to be safe if it is found to cause cancer when ingested by man or animal or if it is found after test, which are appropriate for the evaluation of the safety food additives to induce cancer in man or animal.

There are three Delaney clause is one for color additives and one for animal drugs and they are in essentially the same for our purposes so we focus on the one in the Food Additives Amendment in 1958 and you can ask yourself what is in the Delaney clause that is not covered by the general safety class? Is the general safety clause says it cannot be safe means a reasonable certainty and no harm that's why we need something that explicitly addresses cancer. There was increasing concern over cancer rates in the 1950s and to the parent agency FDA at this time objected to the Delaney clause because they saw it as unnecessary and it didn't add anything that wasn't covered by the general safety clause. To get the amendment passed at the end of the day there had to be some compromise to satisfy the push for some specific reference to cancer, so we got to the modern Delaney clause, which actually has more flexibility built into it than what some of the initial attempts to specifically mention cancer. So the Delaney clause gives us a bunch of questions each of which we could spend an entire seminar, so when does the substance induce cancer? There is a point of interpretation and flexibility. Which tests are appropriate? Another interpretation of flexibility. What do you do about carcinogenic constituents? The Delaney clause targets the additives; but what about all of the little things that might be present in that additive? In addition, that the Delaney clause that does apply to GRAS determinations? Does it apply to agency-initiated rulemaking? We have to think about the process whereby somebody submits a submission to approve a food attitude but FDA of its own initiative can start rulemaking to approve an additives and the detainee clause is not placed in that provision of the law. It is a place on the provision that talks about people submitting petitions.

That is as much as I think we can say about the Delaney clause and I will mention it again in the context of the cases that it but back to the question of allocation. The FDA makes it clear that Congress clearly places at the burden of safety on the sponsor of the food additive petition FDA does not have to prove that the product is unsafe. So you have a great area and the petitioner has the burden so if the regulator is acting to take something off of the market and has the initial burden of showing that there is on safety question.

Finally asked that I would discuss more about fair evaluation standard and these are the words of that FDA uses. There must be an objective basis for the evaluation of the data presented for a scientist to conclude that the substance is not been shown to be safe. The requirement of objectivity is met if the agency reviews a fair evaluation of the head is evident state's reasons for crediting or not crediting a piece of evidence ways all of the evidence applies the correct statutory standards and decides. That is a lot of words to capture the notion that we need to have confidence that the evaluation has been comprehensive weather in the context of a petitioner coming to FDA to get FDA to approve something or in the context of FDA acting to provoke he broke a prior it approval.

We also have to mention that we had the procedural safeguards in place. I mentioned in another comment rulemaking. This is how the food added to approval process is different if the FDA first publishes a notice that the petition has been filed and the food additive has been approved. It publishes an order and any person adversely affected can file an objection and a public hearing, and if the FDA finds that a substantial risk has been met we meet with a formal hearing process to get all of the issues on the table and get all of the evidence in the record. Then FDA is acting on any objections subject to judicial review so that is our safeguard in our system. We have the executive branch making the decision about a food additive and if a petitioner is unhappy with that decision and thinks it doesn't meet the standards they can step outside of the executive branch to request review of the judicial branch. When the judicial branch is reviewing the finding of facts will be so stained based on a fair evaluation of the entire record and as we will see, challenges can take years and years to resolve.

So here is our first study. Approval contingent upon labeling, I thought that this was an interesting one because if you are talking about extra scientific factors that get into this question of what is an objection. This highlights the dual nature of food approval versus a GRAS is so this started in 1987 starting with the food additive position for its use in food, which triggered an eight year, review. This was an unusual situation because we had a potentially something that was going to be consumed in fairly significant quantities so FDA undertakes a review and pulls in outside experts in the form of food advisory committee which does its own review and makes a recommendation to FDA that it can be approved under conditions of intended use. In 1996, FDA goes ahead and him proves some uses contingent on a warning state went and review of post-market studies. Fast forward a couple of years the food advisory committee reaffirms its original recommendation and if additional evidence comes out and allowed for a reappraisal of that warning requirement FDA decides a it is not and in 2003 eliminates that reporting requirement. In 2004, FDA approved additional uses for now that petitioner comes in a wants additional uses and then in 2008 the petitioner says we have other uses for which we think we can meet general recognition standards are not only do we recognize that they are safe. For those uses we will submit a GRAS notification so that comes into FDA and FDA issues a letter saying they have no questions about the petitioner so you have a example of labeling of the condition of the intended use and you have it submitted for approval and GRAS and it enfolded into it and that is nice.

A second one to show you is the Delaney coming into play so this has been in use for saccharin was used for quite some time and it was one of those that FDA has to decide what to do with it and FDA included it in a regulatory listing of grant substances back in 1958. Along the way science developed and you have some studies suggesting there is a potential safety issues. So FDA pulled back on grant status. So we have a “we don't think that we will say it is unsafe but we have a question,” so we provide for interim food additives into the regulation. So forward to a 1977 study reveals a bladder cancer and FDA says our hands are tied, so if it in this is cancer in man or animal it cannot be an approved. Given now you cannot have the legislative branch stepping into the frame saying, “we cannot allow saccharin to come off of the market because it is a non-nutritive sweetener that diabetics depend upon” and a number of others have already been taken off the market. So there was a lot of interesting concern of the impact of the saccharin so Congress imposed a moratorium to let us gather additional evidence. In the course of succeeding 10 or 15 years you have enough evidence coming into play to say what happens in rats will not happen in humans so based on that in 1991 FDA from the scientist perspective the report in 2000 taking the list of saccharin off of the list of carcinogenic substances and it was removed.

The last one is abandonment of challenge uses. APA reference in earlier discussion and for quite some time before the concerns of safety of the contemporary reviews we can see that consumers have submitted a petition to FDA asking for a ban on BPA. FDA imparts on a four-year review of safety and

meanwhile the petitioner is getting tired of waiting for an answer. So to FDA to compel a response going in the judicial branch to say the executive is not doing what they're supposed to do, which is enforcing the law and we need to use that force them to make them do that. In 2012, FDA denies that position and the issue becomes a moot because all of the manufacturers are running away from BPA as fast as they can where there is sensitivity on the part of consumers. So there are still reviews ongoing but from the safety purse effective the market place still reacts and you have what is an effective of the abandonment of the contraband seal uses.

So to close out key points. The determination of whether an effect is adverse is only the beginning of the discussion and you start to put these things into a regulatory framework. Second is the determination of adversity has to be placed in light of whatever the governing standard is some type of adversity may be under one standard and not under a different standard and that sets a different balance point. Third the likelihood of controversy depends in part on the quantity and quality of underlying evidence with a big asterisk. Where before we had a more controlled environment for the determination of scientific information, now we have open access journals and we have bloggers. All types of things that can take some findings and studies that might have met anybody's ideas of standards of qualities and all of a sudden it catches the public of imagination becomes a mission an issue that everybody has to deal with.

[Captioners transitioning]

Where there might be market forces at play there might be unintended consequences that have helped the occasions at play that can cause either the legislative branch to step in or some other corrective mechanism to be triggered. The last point is the pace at which science evolves argues in favor of flexibility and discretion, which is as you can see from the very early days FDA has worked very hard to try to maintain. We have those limits that we talked about in the procedural requirements and so on but what we still have is a very both a robust and flexible system for evaluating safety. A lot of different context and I think we're about out of time in our scheduled for a break if anybody has one or two questions they want to ask now, happy to take them otherwise we have the panel discussion later.

[Applause]

Audience Question 1
Thank you very much; my question is in regards to the Delaney clause. The word appropriate in legal some point would you consider appropriate let's say exposure in somebody has inhalation study and are you going to use the inhalation study [Indiscernible - heavy accent] [Indiscernible - Poor Audio] appropriate based on somebody is doing that confusing for me from her perspective -- [Indiscernible - Poor Audio]

Richard Carvajal
You pointed out a beautiful minefield I will not step into, but I will say this, when we say causes cancer in man or animal, and very clearly ingestion is something as considered a prerequisite. Once you get past that and once you are dealing with the world of ingestion. I think there's on the one hand the rigidity of Delaney that says it is bound to induce cancer you are done. On the other hand there is the flexibility in the use of the word appropriate, which has a lawyer, I love anytime I see. Because appropriate is of necessity something that will reflect whatever the current understanding is so whatever is the best available information is something that we can draw on and what is considered appropriate today I would expect would be different than what might be considered appropriate 20 years ago will be considered appropriate 20 years now as scientific understanding evolves. Feel free to jump in.

Audience Question 2
I have another question [Indiscernible].

Richard Carvajal
I see that others recognize the minefield – [Laughter]

Audience Question 2 Continued
Very nice presentation, we've heard a lot about new technology, new approaches to risk assessment approach and toxicology. Would you comment or maybe think about what this means within the context of the grasp -- GRAS general recognition some uncertainty with the new technologies and new or technology what does that mean in terms of the GRAS concept and the kind of technology – [Indiscernible].

Richard Carvajal
That's a great question. We talked about GRAS handling those two elements and the discussion leading up to this including one we had last time and you will see there will be more little later in the second half of this. We've really been focused on some of those new approaches that when does the evidence regenerate for those new approaches become something that a regular can rely on to make a safety determination? That is a threshold question, if we are comfortable that those things have achieved a level of validation for lack of a better word recognition with the scientific community as being legitimate ways to speak to the safety we are part of the way to GRAS status. General recognition there has to be enough out there and end up available for both, had a chance to digest it and really weighed in on itself. That something are safely personality context of a particular food additive petition and really hasn't filtered down into the scientific community, then I think that there might be questions about whether a general recognition of safety based on something that has achieved such low visibility is something that would be appropriate.

Audience Question 3
Hi this is Mary from FDA, you covered a tremendous amount of territory in a very short time and is been quite clear and good in one thing I wanted to mention, was having worked on Olestra. Even though it is widely believed that the labeling was a warning statement, the agency said it was in information statement, as this as being novel ingredient and consumers had no experience with it just – [Indiscernible].

Richard Carvajal
An important -- everyone heard that important modification –

Bernadene Magnuson
Unless there are burning questions, I think we will go for a break and please be back on time at 10:30 AM. We will start promptly at 10:30 AM -- thank you.

[Applause]

[Event on break until 10:30 am ET.]
Sabine Francke
Please take our seats now that your seats now – [Indiscernible].

10:30 AM–11:10 AM No Observed Adverse Effect Level: Sucralose as a Case Study
Bernadene Magnuson, PhD, ATS, Health Science Consultants, Inc., Mississauga, ON, Canada

Thank you very much and welcome back to everybody online as well. The topic of my presentation is no observed adverse effect level and I will give you a case study. The first slide is very detailed Conflict of Interest and won't read it all basically, it comes down to this is my scientific opinion and I have no Conflict of Interest.

What I will do today is step through the process of an approach to the assessment of adversity for a novel food additive using the more traditional approaches that have been used and discussed within that what are the various considerations that are taken have it taken in establishment of the no observed adverse effect level and specifically the example. Today it is sucralose. What I want to emphasize is that what I'm talking about the consideration for determination of adversity, this is relevant whether or not we are talking about establishment of the no adverse effect level or as was discussed by Nigel whether we are talking about using a different modeling approach using the BMD. So I will highlight the importance of the GLP studies which has been referred to in standardized animal methodology testing in terms of how that facilitates the consideration and a little bit in terms of consideration for susceptible subpopulation, new research and how that is being integrated, and then of course the whole question of human relevance.

Just a reminder, that the ultimate goal which has been set by numerous people is that the whole goal of determination that we are tightly about today is to ensure the proposed uses of whatever food ingredient that we are talking about here or food additive is safe for humans. The scope of that in terms of young, old, and so on, is very broad. Then I want to spend a moment talking about the definition of no observed adverse effect level or the NOAEL and put this into context. If there's two different things we are going to discuss today, one is for a specific study and here it can be defined as the dose determined by empirical study which no adverse effect induced by the test article are observed. Again adverse effect we will spend a few minutes talking about that definition but including any harmful anatomical biochemical or functional changes in an important point I want to bring in here is that there is opportunity as was being mentioned that there is an important point of expert opinion, expert judgment, and reliance on past history and so on. So it is not only a statistical consideration, and that some harmful effects may not necessarily be statistically significant while some statistically significant effects may not necessarily be harmful. So that is what we -- part of what I will give examples of as have been illustrated in the case study.

The next step is to integrate that information from the various studies and establish the no observed ASPER effect level for the ingredient or test article. I will illustrate how that is coming from multiple studies considering various endpoints to define the most important adverse effect in the most sensitive species and of course again. The reason that this is important for an overall ingredient is because this is often the reference point that we used for establishing a health-based value such as acceptable daily intake or tolerable weekly intake and so on. That is why this whole concept of establishing the no adverse effect level for the ingredient in general is important.

Moving on we have a variety of definitions and that's a discussion we are not going to have today in terms of what is the best definition, but I wanted to highlight this one because I think it brings in a couple of key points that I will further discuss today. It is a test item related change and as we go through that is often not obvious, so determining that the effect actually due to the test item change in

various endpoints is important. Secondly, that that change results and impairment of either the functional capacity to maintain homeostasis of the organ, organism, population, or in impairment in the capacity to respond to an additional challenges. Again this is pretty wide definition and trying to break down in terms of some of these different aspects in how what we have to consider to make that determination of an adverse effect is what I will do and talk about.

I want to talk through the stepwise process that is used and some of the challenges that we are facing in terms of determination of what is an adverse effect that is being induced by that test compound. In the first step, again it's a very nice paper I've given you the references at the end you can refer to -- is the initial screening process so that can be quite involved in terms of really understanding the chemical characterization of the compound under question. That can be quite straightforward if we have a highly individual compound in much more complex depending on the type of ingredient that we are actually looking at and whether we are using for example an entire ingredient a whole food and so on and *in vitro* method in terms of using for screening process and predictors. As we talked about can be very useful and in terms of *in silico,* I'm talking in terms of structure activity relationship that can be used in predictors of protein allergenicity and so on. We can use and various *in vitro* methods that for example genotoxicity studies, are all used in early on in the staging early -- step process. For food ingredients we have an additional question in terms of what is the impact of digestion and what is the potential that is actually then going to ultimately -- internal body going to be exposed to. Stability in food matrices in case we are talking about what is going to be happening to that food during when you put that ingredient in and what is the stability during normal storage conditions and what is the stability during food processing baking etc.

Whether or not there any other components that are formed during those processes, is also part of that initial consideration and I want to point out here that this point in terms of being able to improve our ability very early on in the process to identify significant predictors of adversity. It can be very helpful in terms of assisting the industry for example and agencies -- in terms of what are the compounds that further resources should be invested in for further product development and further safety assessment testing. The next step is moving into tolerance and in ADME and I will not spend a lot of time on that. Just again to revert to previous: well that does been presented this one that was on ADME for anybody not in this audience but online. I can refer you back to one of the challenges we have is that for safety assessment of voting agreement the protocol is that we are adding that ingredient to a diet that is suitable nutritionally for the species that it's being tested. That can be a challenge for example it can affect the palatability of the diet, and effect food intake and we will discuss that a little more. It also can affect in terms of the diet formulation can be quite complicated and we were just involved in approval of a new serial for human food use in Canada. You can imagine when we are talking about that kind of ingredient and adding it to the diet then you have to look at compensation and adjustment for all the other nutrients that are being added in when you are testing that food ingredient. This is especially challenging when we talk about ingredients that have the potential for use at fairly high levels in the human diet because what we need to be able to establish his safety at a high enough level in the animal model. So that we have that margin of exposure so that we can apply the safety factors that have -- were discussed previously to establish an acceptable daily intake at a level that is actually useful for that ingredient in the food supply.

Part of this then as again to use ADME to you identify the species most similar to humans. The toxicology studies -- of course that is the crux of a lot of this, and just in terms of the number and the types of studies that are recommended, are going to depend on both the type of ingredient, what are uses and thus likely potential exposure -- but also the type of compound itself. For example, often the different Cramer classes are used in domination with potential exposure to determine the level of

concern and that will determine the types of studies that are going to be recommended. Again in past colloquia on the Cramer classes and the questions we want to address of course is exposure affecting any help parameters through the various life stages. If so at what does -- and as you all know this requires an extensive amount of resources and time in order to do this and that of course is part of what is driving this force is being better able to do predictions and avoid investment in studies that are not necessarily going to be used.

Once we have established those safety levels, in animal studies there may be human studies conducted to again confirm tolerance, as expected human expects her exposure levels and to identify if there's any susceptible subgroups that require special consideration.

Now I will move into the Case Study of sucralose and this of course is a high-intensity sweetener that was discovered in the late 70s. It is a [Indiscernible] in which three of the hydroxyl groups have been replaced by chlorine and that change of those three guys those three positions has significant impact on the properties of that compound and first of all sweetness increases by a factor of 600 so it is 600 times at the sweetness of sugar. It also means it is resistant and is not digested into monosaccharides. It is not metabolized for energy and that makes it non-caloric. Some characterization is that it is highly stable in food and beverage matrices during storage and it is not broken down during typical processing heat processing and baking of food, which has allowed it to be used in those. I want to make one point in terms of the implications or the significance of this 600-sweetness level. It's an important point I think many people do not think about that when you take your sugar packet of sweetener and start to actually put it into your coffee. That actually contains only usually 1% of sucralose and 99% of filler because of course you cannot really very easily measure one very easily measure 1/600 of a teaspoon. So it is not a consumer friendly application so a lot of people have a misconception in terms of how much is actually in here and I will mention again how that has been an issue for some of the toxicology studies.

So just briefly, in terms of toxicokinetics there's initial screening that is not digested hydrolyzed I anyone million ends up mammalian enzyme it's been an extensive data set -- five species were assessed for the toxicology netted. All of them are similar but the most similar one to humans is the rat and again that has importance in terms of choosing the most appropriate study later on. In the overall summary from all of those studies combined, illustrate that it is poorly absorbed in all species only about 15% is absorbed in humans as some individual variation in the remainder moves through the gastrointestinal tract unchanged and treated in the feces. As I said no digestion or metabolism in the digestive tract it's actually very little the tablet that is absorbed very little is metabolized in the liver so the overall picture the majority goes unchanged into the feces and that is excreted in the urine either sucralose or neutron metabolite.

So there's a variety of spectrum of toxicology studies that have been conducted and it is the accumulation of the evidence of all of these different studies that are used to establish the overall no observed adverse effect level which ultimately then will be used to establish the acceptable daily intake. I will give you highlights from some of these different studies in terms of consideration in determining whether or not there was an adverse effect and at what level.

The first consideration I mentioned is whether or not the effect actually is due to the test article and often that seems like I've added it to the diet so therefore that is what is different from a control group and that was my be what cause effect but I will illustrate that other factors often have to be considered. One of the advantages of using and one of the principles of course in the guideline they studies is that

there are multiple doses that are needed. That whole point is that once you are able to see an increase in response with those that gives you much greater confidence that the effect you are seen is in fact due to the test article. It helps you to understand that relationship between the change in the effect and does and importantly of course gives you the range at which that effect was not observed.

Also important is whether or not the response is different from the control group -- again often simply the statistical analysis is insufficient to really clearly answer that question. There can be as we have been talking before quite a wide range in the natural variation of a particular endpoint. Although you may have a statistically significant difference between your test group and your control group, if they are all still within the normal range of variation for that endpoint, that would likely argue against the fact that it is potentially due to the effect of the compound. Again comparing it when you have something, which is a [Indiscernible] useful talking about the studies comparing to the historical control data to get a broader understanding of what is the natural variation. The question of biological plausibility -- again comes into play here. And the use of course is very effective or important when we are TACOM about establishing it for the compound in general is whether or not this effect is consistently observed in multiple studies, over a variety of species and whether or not it is one or more or both sexes –

Again it was mentioned before in terms of the importance of conducting good laboratory practices in order to be able to go back and really look at where there any other factors environmental factors or other considerations in the conduct of that study that reduces your confidence that the effect was in fact due to the test article.

One example here from sucralose and again I'm using sucralose because I think the consideration for whether or not the effect was adverse or not or very well documented and described in the publicly available literature. Consistently among both rodent species and throughout various studies, there was a significant reduction in body weight when sucralose was added to the diet and that was accompanied by a reduction in food consumption and there is a dose response the highest effect being at the highest level tested which was 3% of the diet. So the question was is this reduction in body weight which was significant and deemed to be an adverse effect deemed to be in important significant reduction in body weight was that actually due to the reduction in food intake, or was it an effect of sucralose itself a direct effect of sucralose. So that led to a whole subsequent series of studies trying to answer that question. One was the same doses of sucralose were administered by gavage which would then eliminate this effective palatability of this sweet compound on the food intake. There you had the gavage administration did not affect the food uptake and the other approach was to use pair of feeding bearbaiting to assess what the reduction in food intake was solely responsible for the reduction in body weight in this was a very complicated analysis. Here I think what is important is that you have different interpretation of all of those studies by two different experts groups and to different conclusions. So WHO Joint Expert Committee concluded the reduction in body weight was due entirely to the poor palatability of the diet and the low food consumption. Based on the evidence from these studies done in gavage and their feeding and they concluded then that the chronic rat study the pivotal study no observed effect level then was the highest dose tested.

US FDA made interpretive data differently and they concluded that the reduction in food consumption accounted for the reduction in body weight only up to the level of the 1% in that was one percent and then the next dose tested was 3%, they concluded that that was not sufficient to adjust for the change in body weight. Therefore they concluded for the very same study the same data set that the NOAEL was 500 mg per kilogram in this illustrates the importance of interpretation and secondly now moving to wants you determine the effect is in fact due to the test article the second question is that change

adverse or not?

It's been a discussion in terms of wind changes observed and attributed to the test compounds, would be considered non-adverse and I'm not going to necessarily go through all of these but I will highlight a few. -- Terms of adulteration -- there is no change in the function of the test organism or effect -- affected tissue there's a concept that we talk about adaptation and it within the normal range of response whether or not it is transient, whether or not it is limited below threshold of concern. The question of threshold of concern is appropriate when that has been established such as body weight, and for example anything less or anything greater than 10% reduction in body weight but for many effects we don't have an established threshold of concern. Whether or not it is isolated whether or not it is precursor lesion a secondary consequence and whether or not the fact arises from an inherent biological property of the animal model and that is going to be discussed a little bit more in the next presentation fashion example of that.

Moving back to our example of sucralose, we had a situation in one of the 26 weeks studies where there was an increase in the kidney weight and that was again the dose response relative and becoming significant at the high dose group. That was considered in terms of no changes to function and no change in tissues. Kidney tissue morphology take on the pathology evaluation and no effect on the function of the liver of the kidney based on no change in plasma electrolytes. That was determined to not be an adverse effect in the establishment of that global for this particular study.

The adaptive response -- throughout all the studies up two of sucralose at the high levels there is a dose related enlargement of the count in its consistently observed regardless of species. This was considered in adaptive response based on extensive literature from other compounds as well that it is a physiological adaptation to large amounts were we have in this case at the higher doses of poorly absorbed dietary components. So again, the change in the way and enlargement of size of determined to not represent an adverse effect but in adaptation.

The next that once we have an established -- was looking at whether or not the question of susceptible populations -- that diabetic population considered to be a special population for two reasons. One of course is because they are likely users. They are likely high users of this ingredient because it does not affect blood glucose and again because of their particular difference in terms of glycemic response. So prior to approval there was a series of studies that were conducted specifically to look at the effect of sucralose in this particular Target population and again looking at various parameters like glycemic control bled insulin level flood glucose levels CR peptides sea levels and so on -- continuing in terms of the question of susceptibility was addressed early on. This then led to the overall totality of the evaluation and the established known observed effect levels for sucralose was based to be the primarily driven by the study in rats, which was an appropriate species, based on the toxicokinetics. Also they had the greatest life span of greatest proportion because exposure began *in utero* and extended for two years. It was considered the most appropriate one and therefore the acceptable daily intake was reflected as being 100 fold of the NOAEL. I will talk briefly in terms of -- since he approval of -- there has been ongoing -- new research has emerged that has raised new questions and addressing endpoints that were not previously assessed in the early approval process. So one of the new developments is the recognition of pace receptors and we all know taste receptors in the time can detect these sweet compounds and activate sweet receptors and send a signal to the brain and you have this happy face that everyone enjoys sweet foods. That is the role of taste receptors in the intestine and what we now new research has shown the same molecular taste receptors are actually present in other tissues in the gastrointestinal tract, which do not necessarily sent the signal to the brain. That --until there is no

perception of sweetness by the brain no happy face here -- what is happening is there's activation and these have been well established -- to be activated by glucose and other caloric sugars. The question -- this leads to the release of gut hormones that has downstream effect in terms of the emptying in terms of insulin release. Potential effect on sick idea especially as there is now increasing concern of obesity that these kind of questions are raising a lot more interest in terms of understanding the nonnutritive sweeteners stimulate these receptors and at what levels and if they do what is the functional significance of this interaction at that level?

This has led to studies again for a number of nonnutritive sweeteners and sucralose is one of them that has been investigated and *in vitro* studies have in fact demonstrated that sucralose does interact with these intestinal receptors. Ten that can stimulate the release of these gut hormones and there you have similar to the paradigm or the continuum that Nigel was talking about we are now looking much earlier on insane just there's a receptor level and there is a subsequent activation. The question is then is what is the outcome of that and what is it significance at the levels that are being consumed by humans and in a human model. That led to subsequent acute animal and human feeding studies and there's been quite a number of these that have been published using different designs with healthy and diabetic subjects. Most important no effect on gut hormones, so overall what we have demonstrated is what has been demonstrated is that there's no adverse effect on the function that are affected by those gut hormones. So there's no change in terms of the ultimate bled glucose response insulin response appetite and got an gastric emptying and I want to point out here too that this has been reviewed in a number of studies and -- confirms some of those early on predictive studies that where we had long-term consumption that demonstrated again that no adverse effect. They have confirmed the human studies even of going back and looking at some of these other endpoints. There's been recent concern in terms of the carcinogenicity of sucralose that has been, from a recent study. So prior to approval, there's extensive work on the genotoxicity in of sucralose showing it is nonnutritive and it in most studies mutagenic and some that guideline compliance carcinogenicity studies conducted again under GLP, conditions and both demonstrating no treatment related change in the [Indiscernible].

In contrast, to the recent paper by Dr.'s [Indiscernible] Institute has reported that their statistically significant increase in hemopelagic lesions only in male mice and not in females. I won't get into that discussion in terms of the specific statistics there. They have been now these results have been reviewed by a number of experts of course because this raises some questions and overall conclusion is that the reliability of this study is questionable for a variety of factors. One is the study condition and how it was actually conducted is not clear often from the public that publication there is been a long history of chronic infection in the animal comp in his from this laboratory that have been associated with the incidence of these same exact tumors that are being reported. So the question is -- chronic infection there's been questions raised about the validity or the reliability of the pathology diagnosis and statistical analyses in that has been the result of NTP pathology working group reports that is publicly available and so on. In the end, this is also under review right now by and in terms of trying to get more information and what is available from that publication to actually be able to determine whether or not this is a reliable study. I am now going to move to the question of whether or not the revolt that relevance to humans and that is the endpoint we are most interested in.

One of the points I wanted to make here is going through this process of the weight of evidence that is sufficient in an animal model to really establish a mode of action. Again there have been discussions in terms of all of this in previous colloquia but are the results consistent with all data available and as I said before clears those response in getting back to consistent with all data available. One of the questions or points I want to make was -- with regard to having clearly appropriate controls has sometimes been a

challenge in some of these studies with sucralose. The test agent that was added to the diet was not pure sucralose but rather the consumer formulation that contained 99% of the filler substance and when that is not adjusted in the control group. Then it is very difficult to determine whether or not the effect actually is due to sucralose or whether it's due to the filler and whether that only really becomes a significant impact on the diet when you start adding it at levels of 100 or 1000 times what is going to be in the human exposure level.

Again going through the motive action and trying to identify those key events that are occurring and whether or not they are altering to a level such to stimulate the next key event to ultimately end up with the adverse effect is the whole concept of mode of action and adverse outcome pathways. So once, we have that established then in the animal, the question is it biologically possible in humans? -- Do we have, for example, it happening in an organ that is not present in the human? Is there some other physiological or biochemical differences that is very much were differences in terms of metabolites may come into play that may not make that information not biologically plausible in humans. If the answer there is yes, then we start looking at other differences in kinetics, dynamic factors, and so on and again this is all being discussed previously in terms of whether the whole adverse outcome pathway are only high doses needed to activate this response, and how relevant is it to human exposure.

I can see I am almost out of time and I just want to finalize what the summary in terms of the consideration of whether or not something is adverse and as has been mentioned this is often very challenging because there is a lot of information that is coming out from peer-reviewed studies. So on where it is not always enough information to be able to clearly establish whether or not the effect that's been reported. It is clearly being statistically defined but whether or not that effect really can be considered to be an adverse effect ensuring that, it is due to the test compound entering the proper negative controls and that you're looking at any other environmental factors. Again within the context of the study and looking at historical controls, severity of incidence correlation it's important to have opportunity for expert judgment and to consider biological significance. Not only statistical significance so the overall weight of evidence consistency of cross studies in biological probability are things I think are consistent throughout all the talks you will hear today. So with that, the reference list , if there's any question specific to my presentation, happy to entertain them otherwise we will hold the general question for our panel -- exciting panel discussion – thank you.

[Applause]

For-time purposes we will move on.

11:10 AM–11:50 AM **New Approaches to Adversity Assessment in Food Safety Evaluation**
Daniel Krewski, PhD, MHA, NSERC Chair in Risk Science, Professor and Director, McLaughlin Centre for Population Health Risk Assessment, University of Ottawa, Ottawa, ON, Canada

I'd like to thank the organizers of this excellent session and particularly Dr. [Indiscernible] who invited me to be part of today's colloquium. I think the organizers Berne, Marguerite, and Sabine have done an excellent job in putting together an exciting session. My talk today is going to focus on new approaches to adversity assessment in food safety evaluation and the previous presentations to talk a little bit about how we will transition over time to perhaps new approaches to toxicological risk assessment.

Declarations of interest [Indiscernible - Poor Audio] received funding from the McLaughlin Foundation on a competitive basis from the medical school to establish the schools, and your 2000 Holden Industrial Research Chair in a peer-reviewed University; including three partnerships were from Natural Sciences and Research Council of Canada. The research is my own not that of the industrial partners may also keep scientist for risk science international which is a Canadian company established in partnership University of Ottawa and 2006 we do risk assessment work for public and private sector clients.

I will talk today about some traditional in future approaches and how we will go to transition between these to the theme of this morning's Colloquia is adversity. See that might involve as well I have some material on severities scoring reflecting various degrees of adversity and integrated approaches to assessing multiple data sources which I will describe foresee and finish up with a brief conclusion. So we go back to the 1980s there is a paradigm developed by the Food Safety Council for Food Safety Assessment. A lot of talk when into this all-sequential approach using all the traditional approaches that pathological risk assessment that Bernadene described nicely for you in the case of sucralose. That embedded in this is a heavy reliance on emailing them ties ecology particularly the lifetime feeding studies we use for food chemicals but this approach will probably change a little bit as we move forward.

One of the motivations for change comes from the case study of sodium saccharine Ricardo talked about. I remember March 9, 1977 we conducted the third of a series of two generations of the health protection [Indiscernible - Poor Audio] demonstrated increases in the -- high doses of saccharine resulted in them warning on that day from FDA and the health projection in Canada in 1981, and removal of saccharine from the EPA's list of hazardous substances in 2010. Based on the accumulation of new science -- the key data that resulted in the initial concerns were the lifetime to generation rodent bioassays rats were [Indiscernible - Poor Audio] straight from *in utero.* Following the course of the lifetime the generation, you can see in this particularly large bioassay done by the IDC clear dose response. Note the large sample size there but the novelty in this study compared to the ones that were done in the 1970s that resulted in initial concerns is that for the first time in those bottom two panels. You can actually -- the data I was describing on my screen which synchronized with yours you can see it's very large study and another thing my timer is showing me zero minutes so somebody will have to give me a warning –

The previous two studies one of which we were done showed that up to the 5% saccharine diet clear increase in urinary bladder tumors and you can see the result in this large binding here. If you like large study here but if you look at just exposure *in utero* and no exposure in the subsequent generation, no bladder tumors whatsoever so it wasn't just the *in utero* exposure we thought was the case. If you look at exposure not *in utero* but just following birth, you get exactly the same incidence of bladder tumors as you do with combined prenatal and postnatal exposure. So we learned a little about the mechanism in your exposure was not that critical factor is actually early life exposure immediately following [Indiscernible.] That was critical the other interesting piece of science in the saccharine story -- if you look at the NTP report the mechanism by which saccharine causes these bio tumors has been elaborated in some detail. Basically to oversimplify you don't need to read all the words in high doses. You get information of microcrystals in the urine, which irritates the bladder wall in cell regeneration, and ultimately bladder tumors are formed if you don't get the saturation the microcrystals you don't get the risk so the relevance to humans is kind of discounted by this finding from the NTP.

That's a little bit about the past and I wanted to show the mechanism by which saccharine induces urinary bladder tumors. As the heralding of this new approach of toxicity pathways, which we

elaborated in the NRC, report Toxicity Testing in the 21st Century that will come to shortly. Here is a paradigm from a 2014 publication based on a multiyear project led by the US Environmental Protection Agency to chart the future of the science so we worked on this for three years at a number of public sessions. We had consultation with over 200 groups and individuals during that period and we came up with a paradigm that basically tried to incorporate all of the new scientific tools and technologies that we have broad toxicity pathways into the traditional four-stage risk assessment paradigm and you can see the four stages that Ricardo talked about. The risk assessment paradigms are indebted in there. The two overall changes and it'll be the final metal contribution of the first in the three cornerstones, which is the Toxicity Testing in the 21st Century, were. Thus also advanced risk assessment methodologies used to interpret the data, which I would describe a little bit -- talk for risk assessment. It's embedded in here but that is one of the contributions made in our own center in advancing science -- so let's go back to the first of the three cornerstones, which is Toxicity Testing in the 21st Century. In 2004, an NRC Committee was convened to look at how can we take advantage of new scientific developments to enhance the way we evaluate toxicological risk. We were given carte blanche, there's a cabinet you painted the way you think the future should unfold and an opportunity. But what a challenge because the scope is unlimited and eventually came up with this paradigm. To oversimplify the key is let's reduce the traditional targeted testing approaches of the type you saw in the Food Safety Council paradigm I showed a few slides ago. Focus on new ways of identifying critical parts of toxicity pathways, and focus on establishing exposure levels that will avoid those pathways or division and that will be a new paradigm for safety assessment. This does not require that we track results all the way through adverse health outcomes so [Indiscernible] outcomes -- and since that report came out we have built a series of articles which describes in some detail how you might implement this key in the new approaches. Are the use of new high throughput *in vitro* testing procedure the new competition methods and toxicology author tools and technologies to characterize a potential risk and perturbations in ways differently than we were doing by reliance on one million toxicology. Describing dose response relationships pathway perturbations more so than for a typical outcomes and there's also a component, which is perhaps not fully appreciated within the NRC paradigm for T21C. Let's see what we can learn from population base that is a line between molecular toxicology and genetic and molecular epidemiology is blurring and we can often do is study in the human population with molecular and genetic epidemiology that will inform understanding of key toxicity pathways.

The motivation for change in the original NRC report was, we are exposed to thousands and thousands of chemicals in our environment albeit many low exposure levels, not all have been subject to the same rigorous testing as the food additive would be. On a subject so how can we increase the coverage of the large number, we might anticipate exposure increased throughput to test all the chemicals in a cost effective manner. That's more of a motivation for environmental exposure include chemical side the same technologies could result in making earlier decisions whether a new chemical might make it all away to regulatory approval. We can test directly with the *in vitro* tools we have at our disposal in human cell was possibly generating more human relevance and voted toxicity studies some of these new tests are very sensitive. So we can test lower dose levels and get more sense of results, direct measures possibly only down to human exposure models and we can understand toxicity pathways and perturbations and interactions among pathways in multiple exposure. So many more motivations than just the original one have an increased throughput for large numbers of agents when we look at this more broadly.

This is what we saw with various talk and case study of sucralose and detailed in the new approach. If we are focusing on identification of pathway perturbations as the basis for risk assessment may not have information on in a typical outcome. At least to that but we may not even have established that

that pathway privation is directly linked down the road with adverse health outcomes. We will be able to validate this new approach by understanding toxicity pathways which are diverse which are not as we move forward but we will have in the future where decisions may be made else as [Indiscernible].

In understanding how to define diversity this is a slide from a paper by Kim Boekelheide in it relates nicely to Nigel's exposure, to dose, to early biological effect, to adverse health outcomes will be working. More appear in identifying pathway privation of the molecular level personal changes. That persist for some time and will likely lead to down the road and adverse health outcomes but will be working more with the at this point in the continuum from exposure to adverse health outcomes -- ultimately we hope to be able to make the future is dose.

Dose response data in Nigel's presentation, and the benchmark dose as a point of depart STUR, I want to make two points here. There's another point in the departure that we've been exploring the signal noise crossover dose, which is similar in concept to the benchmark dose. The loose idea is you follow the dose response curve down to the point where the uncertainty and response is equal to the variation of background response, so you can't tell the signal from the noise and then you stop, you say I go any further, I'm getting into the noise region, that the limit resolution of the experimental data. We have a paper in environmental health perspective in 2011, which INTS induced this concept.

Since then we've applied this methodology, to 10,000 datasets provided by the US EPA, we've worked with, with senior sign TIFTs from those groups in this publication, which is -- so we fit 15 dose model so over 10,000 datasets representing 1400 different chemicals. There's nuclear receptor assay, stress response assays, this is the new toxicology, based on a large number of datasets. In the paper we actually seek to calibrate what the signal to noise crossover dose looks like in comparison with their traditional benchmark dose, so I'm showing you a schematic of how we're actually doing dose response of indicators of pathway innovations based on large [Indiscernible]. I expect to see more and more of these kind of analyses as we move forward.

I'd like to turn next to a few comments on exposure science in the 21st Century. The NRC report of 2007 was titled Toxicity Testing in the 21st Century. I have to tell you how that title was derived. We finished the report, I'm sitting with the director of the board, and we have to could up with a title. So he says, give me your thoughts. I write down about a dozen titles, and Jim just crosses off the first 11 of them and then he comes to Toxicity Test, that's it, that's what we went with. It was such a catchy title and it conveyed the concept to well, there was a follow-up report, [Indiscernible]. I want to focus on biomonitoring as a key component of exposure in the future we have in both the United States and Canada large-scale biomonitoring programs that look at concentrations of environmental chemicals present in human tissue, we're accumulated large data banks of biomonitoring data that we can use for population based risk assessment.

At the same time, we've developed methodology for establishing exposure guidelines not in terms of ingestion levels but in terms of concentrations in critical target tissues. This is a report of a working group lead by Shawn Hayes in 2008. The methodology for deriving biomonitoring equivalents taking a reference dose based on ingestion and turning it into eye serum concentration, or concentration of other critical target tissue. How you do it since that time, there have been a whole series of biomonitoring equivalents established by US EPA, by health Canada using that approach. So now we can actually match our biomonitoring data, which might be measurements of chemical contaminants present in serum expense the corresponding serum [Indiscernible] that would be deemed to be safe or

the so-called BE. So we're moving now to actually tissue based risk assessment as opposed to external exposure based or ingestion risk assessment.

It gets even better; this is a slide from a paper by Dean Jones, which is just come out talking about high throughput biomonitoring. We have the capacity now to measure hundreds, thousands, tens of thousands, possibly even in the near future hundreds of thousands of analyses in the same tissue sample all in one fell swoop. This is the concept of high throughput biomonitoring. You give me a sample of a serum, and we can run them through this high throughput biomonitoring and it gives me a large out UTS in a single analysis.

Can we combine high throughput *in vitro* testing with high throughput exposure assessment. Yes, there's a nice paper by Barbara Wetmore and colleagues. The idea is in the next slide. On the upper panel, what you see on the vertical scale is dose. The horizontal scale is the series of 163 different talks cast chemicals but the green panel at the top shows the dose at which you see a biological activity in a whole series of different *in vitro* assays. So for a single chemical, you'll see a range of doses, in which you see biological activity in different *in vitro* tests, and the bottom panel is computational predictions of exposure to those agents based on computational exposure modeling which is quick and fast. The point is, in just about every case, the levels at which we see biological activity in a range of *in vitro* tests is higher than the levels at which we project human exposure to be, so we have a comfortable margin of safety across the board. This is allowing us to look at large numbers of agents in terms of biological effect, large numbers of agents in terms of exposure in a rapid efficient way. If all the data, all the analysis we do like this, it's a good news story because we do have a comfortable margin of safety between the levels at which we see bio activity and the levels at which we predict human exposure to be. Just as a secondary comment, we need to understand which of these effects up here [Indiscernible] are adverse. When we're comparing them with levels of new exposure, but even if I don't have a full understanding, of which effects are adverse and which are not, if I've got a really comfortable margin of safety, maybe that's all I need to know.

Switching to another topic, this is a paper, committee report. It was a joint NIH/Health Canada report on setting dietary reference intakes for chronic disease endpoints and the question was can we incorporate chronic disease endpoints into setting a DRIs? That's not what I want to talk about. I want to talk about one of the things that's mentioned in this report is how do we actually handle U-shaped dose response curves for agents which are both essential and agents which have sufficiently high doses can cause toxicity so essential nutrient like copper, manganese, and others. So I would have to model a U- shaped dose response curve now and use that dose response to come up with some kind of exposure guideline. We like to be somewhere in this nice homeostatic region, this called range of normal intakes that needs to be defined I'm going to link this to severity in a second.

So we have a paper, which just appeared in Risk Analysis, which is a continuation of work that's been underway in our [Indiscernible] for over a decade. Previously I would panhandle that U-shaped curve by modeling the excess curve and the [Indiscernible] separately and piece them TOBL. What this paper does is propose a methodology for modeling the excess and efficiency [Indiscernible] it's called we've applied that model to a database on copper Tox-adversity. Copper is an essential element which is also toxic at high doses but the link to adversity is shown in this slide. The technique that we use to fit the J med model, which is category regression, which allows us to look not at specific study and specific adverse outcomes but to assign a [Indiscernible] so we, can combine studies of different long-term acute toxicity on anything you like. By assigning a severity score from one to six, for excess, and from minus one to minus six, if you will for deficiency and the brief definitions are shown here. So we might

have some altered metabolism, we might get irreversible effects, mortality. The concept is you can define an ordered severity outcomes that will allow you to integrate every toxicity study that you've know a single analysis with the severity as the end point.

The data says we have, the numbers in brackets represent the total number of data points that we've identified that are relevant for this U-shape dose response molding exercise. Based on our most recent literature review through about 2011, 2012 is when the data cut off, but you can see for both excess and deficiency, excess is in the top panel, deficiency is in the bottom panel. For a variety of different species, we've got quite a few data points for every one of these severity levels that we've got in our severity-scoring matrix. What we've done here is done a systematic review on the world's literature on copper response to pull out from that literature data points that are informative with respect to dose response and then assign a severity category to them.

This is the database that you can now analyze in a single dose response analysis, and here are the results. This is including every data on every study that has useful data on dose response curves for copper excess and deficiency into a single analysis. I think we used a severity cut-off of three in this particular analysis. We could use other cut-off severity levels 2 or 4, gives you pretty much the same thing but you model the U-shape dose response curves and what you like is identify the bottom of the U which is X, the minute stands for the minimum of the concern. E is efficiency union, excess, and the value is 2.73 milligrams of copper per day, minimize the total risk from excess and or deficiency, and look at these limit how tight they are 1.5. So prior to this analysis I think conventional thinking was somewhere between 1 and 10 milligrams per copper per day is right in the right range. This makes it much more precise and integrates all available data within a single analysis using this severity scoring technique to define on adversity. So just to show you that's not a one off. We've recently done a similar exercise for manganese. It was equally successful, same thing, systematic review of literature. Expert panel to assign the severity scores, we applied our now categorical regression technique, [Indiscernible]. It gets even better in the case of manganese because we did have a validated physiological based model for manganese ingestion. I know dermal absorption and we were able to predict tissue concentrations of manganese in the global [Indiscernible]*, which* is global [Indiscernible]. Which is the key site in the brain for toxicity and we conducted the categorical regression analysis on a tissue dose scale, [Indiscernible]. Did as opposed to external exposure to get an even more precise of dose response.

I have a yellow light, and I've got about five minutes, if that's okay? So my final topic is when you do toxicological risk assessment, you may have data from multiple sources. You'll have classical toxicology, [Indiscernible] you'll have perhaps new data on, toxicity pathway [Indiscernible] as we move farther into the future, and you might have human data of different types. How do you integrate all of that information into an overall analysis? Well, categorical regression is one quantitative tool. That's a data rich tool you have to have many data to a apply it. In other cases you have to make two decisions a qualitatively decision do I have a hazard based on integrating all the evidence and if there is a hazard you may want to do a quantitative analysis. So one the tools I'm going to suggest that's going to be key for evidence integration is a systematic review. I learned about systematic review mostly from my graduate students who took the latest court in systematic review, and told us professors we're not getting our papers published these days unless it’s systematic review, your old style of expert review doesn't work anymore. I was a convert. We had a major project funded by the Public Health Agency of Canada, which was to conduct systematic reviews of 14 priority neurological conditions Alzheimer's, Parkinson's, ALS, [Indiscernible]. It was a huge undertaking, I had a team of 21 people from five research institutions working with this, and we were just completing a series of 16 papers coming out of neurotoxicology summarizing the results of this review. The methodology as described in this

methodology paper, which will be in the special issue of neurotoxicology and I want to go back to that previous slide just for a second. You want systematic review because it's subjective and reproducible. If I do it, if Berna does it, or if Nigel does it, we come up with the same evidence on the table. We agree on the evidence because there are inclusion criteria, quality-scoring criteria. We then still have to make some kind of weight of evidence judgment. Do we have a hazard on our hands? So as a first step in evidence integration, I'm really speaking largely favorably about systematic review techniques, which was quite refined, quite formalized and then if you do have a hazard the final topic I want to mention before I conclude is a meta analysis might be used if you've got a series of studies with quantitative estimates of risk. In this case, these are all epidemiological study on Lou Gehrig's disease, in relation to occupational exposure to lead. And you may see individual studies may not be as precise or informative but the overall all [Indiscernible] estimate gives you a pretty clear significantly increase with risk with pretty quite fight intervals [Indiscernible]

To sum up, we have a new paradigm for toxicity testing based on TT21 C. The NRC 2007 report followed we a new paradigm for exposure science in the 21st Century, that's the NRC, 2012 sequel, and just recently, there was a NRC report on using 21st Century science to improve risk related evaluation. Trying to show how you would use those tools for actual 21st Century risk assessment. All of this change is motivated by a need for increased throughput, lower costs, greater human relevance, and, but the new paradigm that I've described as we start to think about the future will present some challenges in redefining adversity on the basis of toxicity pathway [Indiscernible]. *P*ossibly some adjustment in regulatory practice even though the statutes will admit any kind of credible validated evidence on safety that has been considered appropriate. Hopefully all of these new approaches to evidence integration that I described will help us pull all this data together in a powerful and compelling way. There's a series of references, included in my slide deck where you can get more information on the topics that I described and I'll have to take the advice of my chairperson as whether we're taking questions now or going into panel.

[Applause]

Bernadene Magnuson
Well, I think we are going to move the panel down and start with the panel discussion now. This may be giving us, a couple moments to have our team up at the back of the room to start telling us whether or not there are studies that have come true on-line. While we're doing that, if you want to see if there are any, any --

Sabine Francke
We would like the questions to be projected onto the front here. So we would like to make sure the people on-line that their questions were not ignored that we actually saved them for the discussion now. We will have them projected upfront to be part of this intimate exchange that we hope to unfold. While we're waiting for that, we would ask whether there are any questions from the audience at the same time.

Bernadene Magnuson
I'll take the opportunity to thank Dr. Krewski for his thought provoking presentation. While we're again kind of seeing whether or not there's going to be any questions coming and while, you know, the minds here in the audience are worrying away and coming up with questions, we'll maybe just start off, I'll just start it off here. I think, you know, when we're looking at the types of discussions that we've been having and clearer, you know, emphasis and interest in terms of developing some of the predictive

approaches for defining adversity, certainly can see some of the benefits in terms of, you know, improved throughput, reduced costs. One of the questions that we wanted to have a little bit more comment from the other speakers were really, you know, how are we going to further advance this question of determining when those responses, predictive responses are clearly associated with adversity?

Daniel Krewski
So this is the first question that we got in the first public presentation of the toxicity testing in the 21st Century report at the toxicology forum in July of 2007, and the answer that I gave at that time is still an answer that I'll offer at this point, but I can add additional dimensions to my initial response. The idea behind TT21 C, at least these predictive responses we need to understand toxicity pathways. Understand all the toxicity pathways that operate in the human body. And then identify tests that will efficiently pick up those pathway [Indiscernible] so once we've mapped the human Tox-zone, we understand which pathway period vacations are provision and which are not. This is a big science [Indiscernible] It may take some times we may not answer all those questions as quickly as we would like, but in the meantime, we have known it correlations between the predictive responses, Nigel had a slide showing a good correlation between traditional benchmark doses and transcript only benchmark doses. You've seen our work on comparing BMDs and SNCDs in the large number of *in vitro* datasets that we've been working with, and there's also the work that showed from Barbara Wetmore that human exposures are often comfortable below levels of bioactivity. While they've got that margin of exposure, maybe I don't have to precisely understand which of those bioactivity responses are adverse. But ultimately, we would like to get to a fully validated system for those predictive responses so that there's really no doubt what's adverse and what's not. I'm sure that the other people will want to add to this.

Nigel J. Walker
So there's, there's work going on already looking at current adverse responses introduced on models due in high dimensional [Indiscernible] trying to relate, you know, classic pathological lesions to the biological pathway, so that's one-step in terms of qualitative assessment of which ways pathways are related to specific adverse outcomes, I think the that you can talking about two aspects. One is simply the dose at which something occurs, and the other is what does it mean if something occurs, so you have, to I think logically go forward on both of those arms in concert. One is simply using a, a point of departure in decision making and as part of the, you know, a phase of evaluation, but the other is the research to actually links currently known adverse responses to back to pathways, and identify AOPs and those kinds of things. That's a lot of work. So both can be used in concert with traditional models at the moment to identify those where your margin of exposure is really limited. If you haven't got a good approach, next layer run some additional pathways. Run additional models.

Bernadene Magnuson
Yeah, and I just wanted to add too, in terms of, you know, with increasing youth and increasing evidence to support it. I mean, we have that history of youth for some of those predictive responses from, you know, genetic toxicology, that you know, 20, 30 years ago, with increased use and confidence they are now very well established and very well send. So I think it is again sort of a continuum of use as it starts to be integrated. Any other?

Ricardo Carvajal
I was actually going to ask a follow-up. Motivated by one of the questions I was asked earlier by integration of these new, new methods and frameworks into the GRAS. So I'm just wondering how much

of this is being developed in, as part of the public domain and how much of it is being developed with private investments as a commercial venture. Therefore maybe the methodologies are not out, out there for everyone to see?

Nigel J. Walker
I couldn't give you a quantitative of how much, but I know that one thing that [Indiscernible] do as part of developing new approaches is we have the small business innovation, innovative research grants, which allow commercialization of assays that come out of, you know, innovative and investigative science to move into a commercial center. I can't tell you what proportion of all assays are out there that go that route, but there are mechanisms whereby you can capitalize on research that looks like it could be promising, leading toward, you know, commercial approaches that anyone could then use. Because I think that's also a critical issue of the new approaches is, making sure they're available to company's CROs that can use those when, you know, someone is bringing forward a new product. In the absence of that, it's going to be very, very tough to actually generate that data. So that's kind of part and parcel of why we're helping support at least the development of commercialization.

Sabine Francke
I think that point came up in several of talks. Right, we're talking about assays that anybody can propose. So with that, we open up the field to a very large variety of possible testing strategies, so one of the dog, the other one is interpretation, and that came up in the talk saying interpretation is in the eye of the be holder. In order to makes sense to the entire scientific community and to give us something we can work with as regulators, there needs to
be some sort of strategizing and bringing it back to, to something that is really digestible by all. Right? So opening up the field to scientific thought process, everything is the limit, I think is not very practical. How, how do you feel about interpretation being, you know, subjective when you say, well anything in terms of strategy is possible? We have this problem now, right? We have, this is one of the major criticism of the process we are advocating today in terms of having experience with an obey owe assay and the animal safety testing for almost 100 years and question still struggle with it. How do we avoid the mess TAJZ -- mistakes we have made historically to not be propelled into the future. Talking about a large variety of datasets that need to be streamlined somewhat in their interpretation AL approach.

Nigel J. Walker
I guess, everyone's looking at me. [Laughing] So I mean, I would actually go back to, you know, talking about systematic reviews. One of the things, you know, initially that's been focused primarily on human studies and animal studies, but one of the ways that we can actually gain confidence is applying this the very same sense to these new approaches. You know, what does rigor looks like for an *in vitro* assay? Do we have risk bias approaches for, you know, these new approaches? I mean, I think that's how we can learn from the past. We've come to the point where we need objective assessment of datasets used in very clear guidelines on what's risk of bias? What is, is that particular assay directly, you know, the directness of a given assay? I think applying that as people are starting to take that toward applying it to these new approaches will be away forward on that.

Daniel Krewski
Maybe I could just answer, if I understand the, one of the components of your question. Your concerned about as we move forward -- I'm just trying to clarify one aspect of your question. I think your concern about as we move forward with new types of data, and there's in interpretation, evaluation required to make decisions. How can we be sure we won't make serious mistakes like we made with [Indiscernible] by not testing it in the right animal species in advance of human exposure, and maybe even like we

made with, with saccharine, by identifying something in rodents that may not present a human risk. So two different types of mistakes I guess I'm asking are you concerned that we don't make similar kinds of mistakes with new paradigms?

Sabine Francke
My comment is more, "let's learn from what we have done before we start doing many more new things." Have some sort of a strategy on, on how to harmonize it amongst all users [INDECERNABLE].

Bernadene Magnuson
I think I'm going to take a minute because I thank you for your question. If you can just hold it for a moment because we're kind of ignoring the other questions. The person in the back could scroll up to the top, I saw that there were some -- yeah. Well, right, yeah, you can't read, that's why I'm standing up here. So maybe we'll just -- okay. So there we go, thank you. So the first one is, could the theory on no observed adverse effect level be used in health effects on pet animal species, dog and cats. I mean, basically, to me the answer is, yes, in terms of that really has been in terms of health effects, adverse effects regardless of the species and that is widely used already in terms of various species, so any other comments? All right. Next question is the data on sucralose publicly available? Yes, that's published in studies in 2000. So yes, you can email me if you want it specifically, but all that data is publicly available. And has this information been summarized using evidence-based methods as for judgment. I'm not sure if that was referring to the question on sucralose, all that information was publicly available and also considered during the approval process. Questions on -- oh, you want to take the -- okay. I'll stop reading. Go ahead.

Audience Member 1
Hi everybody. Thank you very much for a really interesting morning. There were a lot of thought provoking things my name is Christy. I was particularly struck this morning with Nigel's description of how at least to some of these new approaches, we're moving further away from what we intrinsically know about the relevance to the human system and as we look at biological pathways or methods. I can weigh data, the relevance of those [Indiscernible] in those assay is more difficult to directly attributes. And then we moved to the, the regulatory in terms of [Indiscernible] and the, the importance of the competent scientist making a determination on whether or not something is adverse, and that's really written into the regulations and how we really do risk assessment. And the concept of GRAS, generally recognized as safe, as these are substances that the society has decided by the use of our competent scientists these are substances that are intrinsically safe. I'm wondering I'm having trouble formulating this into a question. Is there in way, an opportunity here for us? For us to say, "Let’s start to look at some of these substances, real food and generally recognized as safe substances to look at the variance in these?" In some of these newer approaches to say, "Here’s what we would expect as a society for substances that we understand are generally safe." And here's the range of responses that we see? Or are we just opening now a situation where we're looking at substances that we generally know are safe and we have determined are safe and we're going to see [Indiscernible] in these biological responses and now think that some of these things are unsafe. I think these things are two different things that we can do with these sorts of substances and an opportunity or are we just opening risks where before there wasn't any, and I'm just curious on everyone's thoughts on that?

Ricardo Carvajal
I'm only going to start, start to bear outlines of a response. But, I think one of the challenges over the last 30, 40 years has been our increasing our ability to detect, first to detect substances at all, and then in light of whatever framework we're looking at, try to figure out what, if anything, it means that we

have detected those things. I want to loop back around to the concept of intended use, because it strikes me as being critical, right. So it's not enough, I think to look at a substance, you have to think about what use of the substance you intend to have before you could really start thinking about how you're going to approach the safety. So I guess I may, on the one hand, I could see the appeal of using things that are generally thought of as safe for benchmarking purposes. On the other hand, I'm not sure how you're going to deal with what I would expect to be a flood of information that you really are not in a position to do a meaningful assessment of. That's the push/pull I sense.

Nigel J. Walker
You look like you wrote the paper.

Daniel Krewski
This is a really good question. Can we do a thought experiment? Let's imagine a class of GRAS substances which were exposed to at pretty low levels and I'll sake spices and flavors as an example. Let's imagine we run a whole bunch of those substances through the high throughput test. And then we figure out what even the most vigorous consumer might actually consume, and we notice that consumptions levels are way below any level of bioactivity. Is that going to alarm you or is that going to comfort you? I'm throwing the question back at you [INDICATING]

Audience Member 1
Well, [Indiscernible, Poor Audio]
[Laughing]
I think that's it exactly. I think the push/pull that we're dealing with, and I think that's the kind of question. I'm asking so how do we start to define which of those [Indiscernible] represent meaningful changes that we are now aware of that we weren't before. Which of those represent the system and the amount of [Indiscernible] that it generally deals with all the time? What is the normal in the system. And the exposure, the margin of exposure and the intended use are absolutely the fundamental question, but if we start to think of things that the intended use is as a food, we throw kale in there. We say people are eating, pounds of kale. Some people are eating pounds. They're making these smoothies out of it and we throw kale in the system. We see these changes go all the place, does kale become bad or does this tell us that the body is able to deal with more of this change than we might have thought? So that when we throw chemicals in there, we should think about that perspective as well. How much change can the system, how much change is built into the system that we can expect and how do we start to answer those questions?

Nigel J. Walker
I think it's a great question. I mean, I will go back to the benchmarking. You know, both qualitatively and quantitatively. So some of the, the interest in data that's come out of, you know, the initial Tox 21, high throughput screening is the relatedness of chemicals and their profiles. So you actually have a exogamic chemicals looking here. And similar over here and allow us to look into essentially chemical biological space, what pathway disturbances where they place different chemicals. We've actually applied that to botanical extracts, which is getting a little closer to what you're talking about. Where you can actually say, these do this, this looks like that. So I think quality indicates that we can use it to get a comfort level or discomfort level this is looking similar to that I'm comfortable with, or I'm not comfortable with. There are proposals out there of using the dose response to bend things of being hazard non-hazard. I mean, it get to that no adverse effect level and people are using it for, this recent paper from [Indiscernible] and the December time[Indiscernible] had a paper recently about at low levels these are essentially negative compounds and at high levels positive. And you can use that then to start to

validate new approaches. So again it's using the dose response for the, the given effects of being what you use to actually validate a new approach. So it's kind of both benchmark and externally but also benchmarking internally, so I think there's a way of doing it. It's difficult.

Audience Member 2
I think difficult is a very good term to use for these. When you, when you're doing these high throughput studies, one the questions I have is, you know, are you looking, are we skewing our datasets more toward things that are easier? Individual chemicals, water-soluble chemicals when you start putting a lot of [Indiscernible] *in vitro* systems that can gum up the works. So I think one thing to be careful of as scientists is not just to go only for the low hanging fruit and also to consider food, you know? These are complex substances that have been exposed to a lot of different things than your just empty, you know, not empty, but individual chemical so I think it is difficult, but it's interesting.

Nigel J. Walker
While we're moving around – I can address that one real quick. Currently the 10,000 chemical library that's used across the whole Tox 21 partnership is [Indiscernible] not necessarily water soluble compounds people are very aware that the current pool of chemicals that we're looking at in those is a, it's a pragmatic set of chemicals for the types of assays that you can use a the high throughput system currently for. But there are approaches using other systems. Other throughput that has expansion in other chemical space, so it's understood. Good point.

[Indiscernible, Poor Audio]

Audience Member 2
Okay. Over time we've learned about the importance of having guideline studies for *in vitro* tests and I'm just having a, some difficulty with some of these approaches to integrate some of these *in vitro* studies. That may not have been done according to guidelines, and I just want to get, it seems to me like we're maybe doing this backwards and like trying to take all of this information from these *in vitro* studies, some that may not have been done very well. Trying to integrate and make some sense and AOPs out of all this, when it seems like there should be guidelines for some of these critical types of studies that are done to identify adverse outcome pathways. Kind of doing, you know, having guideline first, and then taking a look at that data, and then rather taking a look at this reams and reams of data that has been coming up. Just a few weeks ago we had [Indiscernible] come and do a study and there was a presentation on someone doing just the difficulties of interpreting some of the *in vitro* data. Where some of these concentrations were binding to plates and you know, different chemicals, depending a solvent and basically. I've done *in vitro* studies myself as a scientist and you can pretty much create you want to find by tweaking what's in the medium. So I just would like to get your, I guess, your insights about whether you think it would be better to say, okay, develop guidelines, like OACB has certain guideline for genetic Tox for example, more guidelines and then have, so there would be standardization for People doing these studies in the same manner and interpreting that data?

Daniel Krewski
I would think the National Toxicology Program would have something to say about guidelines for *in vitro* tests, right Nigel.

Nigel J. Walker
Yes. Of course, we do. So I mean, what you're talking about, like, how do you maintain confidence in any kind of data stream. I mean, so if you go back to the early '70s, the, our confidence in the ability to do

rodent based bioassay was poor. That's what got to the peer review and pathology review, question learned from our experience oh, we need to have some level of, be it mere, not necessarily specific prescribed approaches, but understanding where the deficiencies in conduct that we need to are actually put more stringency around. I mean, that's part and parcel of why we have the interagency coordination committee on validation of alternative methods to actually bring together different agencies that might use *in vitro* approaches and actually what do we need to do validate or improve our confidence. It applies across the board what you're pointing out as science moves forward where are the pinch point that we have to increase our ability to understand, what are the bounds on those studies. People have been doing it, you know, [Indiscernible] a lot of people are working on zebrafish lately. There are a lot of assays not necessarily creating standards of how you should do it like you say guidelines of what are the particular aspects that lead to increased variability and uncertainty in the outcome relative to the, you know, what you're actually hoping for. So it's a really good point. And it's something I think that happens as we move forward. We realize that, I can't think of assays but almost like a funnel. You know stuffs comes and gradually you get to a point you get to this is giving us valid information, and you have to make sure the [Indiscernible] is more defined. Part of that is also understand the variability around our existing assays. You know we do on 90-day studies and various assays but we never truly understand the true variability around those currently. We're making efforts trying to understand, like the [Indiscernible] assay, what is the variability around that. If we're now going to compare that to a new battery of assay to look at [Indiscernible] knowing the variability in both is actually very helpful to see what is, what is outside the normal and what is comparable.

Nigel J. Walker
Anybody else?

Daniel Krewski
I have maybe two points to make on guidelines. First, I would agree 100% that there's a great premium these days on guideline studies for regulatory risk assessment. We see this in North America we see this the European union's reach program, so it would be nice to see guidelines developed for some of the new toxicity approaches that are coming on stream. My second comment is almost a question, which I may have to direct your way, Nigel. I have a naive notion that with roboticized laboratories that can conduct some of these high throughput screens that it might be easy to control variability and standardize and systemized as we develop guidelines that a reasonable expectation?

Nigel J. Walker
That's a good point. So I mean, it takes, if you think of risk of bias, and you know, opportunities for exposure misclassification or pipetting errors or variability, certainly when you have robotics involved, it reduces some of that, you know, data can go directly from plate to database. So you know, transcription errors, so certain things we take for granted and why we do GLPs of making sure there's no missed, you know, we could recapitulate what was done, that's a whole lot easier when you have it automated. So to a certain extent yes. That's not to say robotic handling systems don't have their issues with sticky compounds and somebody coding it originally in the wrong way. But it can make it a little simpler.

Audience Member 4
I have a question, which, if we could get back to adversity. Is adversity the sole determination of what's an adverse effect? Is that the sole domain of the scientist and the laboratory person or the manager? And the reason I ask that because it seems like an adverse effect can be very situationally dependent. So there's a societal element in there, a personal element, like a chemical that may produce alertness. I don't want to take a chemical that produces alertness when I'm ready to go to sleep but I'll take it in the

morning. But if you're talking about a food additive that has that component, you don't have that kind of selectivity of when you're taking a chemical, when you're being exposed to a particular chemical in an additive like that. So there's an additional element in defining adversity. It's sort of like an undesirable effect. Which I consider undesirable. And maybe half of the people would consider desirable. And may be the other half people would consider a acceptable effect. That's a component of adversity that I don't hear anyone talking about.

Sabine Francke
Well, maybe I can start from where this whole topic selection started, which is a pathology type of driven definition attempt of what is adverse. And again, in a socio economic way, adversity is again in the eye of be holder. Everyone judges this differently. But when you go to the loose definition which seems like every attempt of a scientific approach comes back to, it focuses on morphology, on a tangible hampering of physiological processes within the organism. So I think a comfort or a preference is not necessarily part of that understanding. Almost would go back to the benefit risk perception that sometimes is advocated in other, like for example, for drugs, but we in food don't do that. We, we focus on what is adverse with regard to harmful. What is not safe. I don't know what the preference plays a lot of part in the definition that at least where I was coming from, putting this, this topic together had a lot of steak, and I want to see what the other experts here think about that.

Ricardo Carvajal
So it's hard to hear someone talk about alertness without thinking of caffeine, of course. And that's not a great example, because, you know, it's already sort of historically embedded in the food supply. Nonetheless even with something like that, you have foods where it's simply declared as an ingredient, so you can readily see whether you want to avoid it or not. You have other foods where it's naturally occurring. One food is associated with the preference of caffeine so that just becomes general knowledge and then some manufacturers go beyond that to put some kind of advisory statement on there for pregnant women for example. Then you have uses of caffeine where FDA has determined that they don't need the GRAS standard, so I would be made nervous by a shift toward establishing some kind of requirement that is based on consumer preference or a desire to avoid, because that strikes me as a bit of a slippery slope. But I take the point that there could be some effects, you know, particularly when you have, you know, substance that has some kind of psychoactive effect. It's hard for me to think again, once I step beyond caffeine, it's hard for me to see how something could have a psychoactive effect. And that not be [Indiscernible]

Nigel J. Walker
Pardon for the misinterpreting some of the question. When something it's going to have a positive negative effect, maybe not psycho activity specifically. I think non-psychoactive ingredients. Take echinacea. People take echinacea because it hopefully will boost the immune system. I think that's a useful similar concept in that adversity as I was, you know, we're talking about early are open, you go beyond what is normal. Normal, normal homeostasis, normal physiology, when you start going outside of those realms, in some cases you get to a true pathological leash on that you can see because the body is growing, an organ is growing, because it's out of balance. And I think that's, so I think to the extent that scientist, as a scientist we understand what homeostasis and what physiology is that some cases it could be one direction or the other, and you've gone beyond normal. So I don't think it's, I mean, I think it behooves us to understand what the bounds are of a response is relative to normal.

In, as opposed to it being a preference or someone's different interpretation. Someone may want to have something that's an immune booster. Some people won't. But at least we have understood this is

outside the realms of normal, so that's why we determine even in immunology, something is SKAS created an immune response, is still a response because it's not within the realms of normal.

Audience Member 5
The realms of normal is another sticky wick it. What is normal of with nutritional status that kind of thing. One additional question. From the standpoint of the Tox 21, how do they account for differences in gender, ethnic, you know, differences, in trying to come up with a safety assessment concept?

Daniel Krewski
So this is one the top three questions that we've been asked repeatedly since 2007, how do we characterize potential variability in the human population of all kinds? And the stock answer that I've learned to, to commit it to memory, is well, if we have enough diversity in the human cell lines that we're using, which may reflect different life stages, which may reflect different genetic susceptibilities. We may be actually able to cover in a high throughput sense a much richer suite of the human population in terms of its genetic and cellular make up than we can with a select group of rodent assays. So in theory we may be able to do betterment I think that theory still has to realize itself but that's a comment that I've often made in response to that question.

Nigel J. Walker
So it's not just in theory that the [Indiscernible] because they've actually found [Indiscernible] in working with the Tox 21 folks with a select group of compounds that was 1,000 genome project where they looked at a whole diversity of diverse panel of different cell lines that represent different sub populations to look at –

Audience Member 5 Continued
The answer to my question, though I think, they have to [Indiscernible] apart of Tox 21.

Nigel J. Walker
Yes. They have to use different cell lines. Yeah. So that's an approach that people have been taking. I mean, the other approach that we're also taking is actually to take it back to the experimental model systems used in something called the diversity model. I don't know if you're familiar with that this is a highly genetically diverse model that essentially is, is balanced in terms of permutations of different traits across the whole genome. So every rodent and every littermate is genetically different than its other littermate. You can basically take 50 to 100 littermates they are essentially are, they're all different. Well, now it was done for genetic mapping of traits. But it could be used to identify molecular events and the genetic bases for responsiveness that you can then take through comparative genetics to the human [Indiscernible] as well. You can't take Tox 21 and a thousand chemicals all at once in many cell lines but many people are moving toward that direction to get to the generic underpinning.

Audience Member 5 Continued
It looks new cadre of viewers that can actually understand all of the --

Nigel J. Walker
Yes. That's quite correct.

Audience Member 6
I want to thank you. I was struck by the introduction today with Dr. Doull and his comment about toxicology, we do toxicology so we can do risk assessments. I'd like to complete that. You do risk

assessment so we can do risk management. And so one of the, [Indiscernible] your presentation, if I understood correctly, you were suggesting that in terms of adversity, with the newer technology, newer assays we're able to assay further upstream before adversity, before adversity actually manifests itself. And as a risk manager I look at that and say, if I begin to use that information, that approach, am I going to be managing the risk at the appropriate level? Do I need to tie trait back, and say maybe I don't want to over regulator over I know habit use of a substance? How do the risk managers begin to understand what the risk assessment based on these further upstream assays mean in terms of managing the risk of society?

Daniel Krewski
That's a really good observation, which has been core to our sort of presentation at the TT21 C paradigm even in the 2007 report, by the way there's a final chapter in there, I think it's chapter six which talks about those kinds of information, implementation, and communication and what does it mean for risk management to some extent. And I'll give you two different perspectives on the question. If I have a high level of comfort that an upstream pathway period vacation is a concern. In other words, that it could left uncheck result in a traditional clinically adverse outcome, than I think you'd probably have a high degree of comfort in regulating on that if you had that connection. So we're hoping that we will be to identify with time in this mapping toxicity pathways exercise or mapping the human Tox zone if you will, identify the critical pathway [Indiscernible] ways that we need to be concerned about, at which point I think Ricardo's confidence in regulating those new types of data will be much increased. I think in the interim I think we probably have some sense of what may be of more concern than others, and I'll fall back also on this margin of exposure approach. If we can identify bioactivity with the comfortable margin of exposure relative to actual human exposures, maybe me don't have to be as confident in what's adverse and what's not because we have this huge safety factor built in human exposure relative to the levels of which we see biological change. While I have the microphone, unless you want to probe further on that. One of the best responses I heard to the question was your response, Ricardo, when you were asked a question; I don't remember what it was. You've said you've laid out a beautiful mind field that I'm the not going to step into. May I attempt you to step into a different mine field, and that is, I know it's early days. I know you told us last night over dinner and this morning during your presentation, you know, that if we have validated new approaches, than we can probably run those through the regulatory apparatus that we have now. But given what you've heard, are you willing to kind of give us your sense of whether it looks like this new approach to toxicity testing that I described in some detail today is probably going to have a reasonably smooth transition into regulatory practice, or do you still see it as a long way to go? Anything you care to say, or not say I'd be happy to listen to.

Ricardo Carvajal
Yeah, I mean, we've heard so much. On one hand, I think there's a real hunger and an appetite for new approaches, particularly in light of what I think is increasing consumer sensitivity in many jurisdictions to the toxicological approach, which is animal testing, and there is an increasing tolerance for this on the reliance of animal testing. So to the extent that we can ground truth alternatives to that, particularly ones that bring down costs are more efficient both for industry and for the regulator, that would seem to me to create a fair amount of, of pressure in that direction. Now, we have these, these ancillary issues that have been raised, such as, well, what do we know about the way that these methods and approaches actually work? How much of that is in the public domain? To what extent is it something that's visible for all so everybody can jump and evaluate and understand exactly what's going on here? And I suspect there are ways of dealing with that problem. So you know from a purely personal perspective I think it's kind of exciting to think about the possibility of integrating this new knowledge base. Do I expect as a practical matter that it's going to happen in the very, very near future in that there

won't be road bumps along the way? Well, you know, we've all seen the kinds of controversies that you can have when you get results that folks weren't expecting. So I think we're in for some of that as well, but on a whole, I would, I would like to see us continue moving along the continuum that we started way back in the late 1800s, seems to me we've come a long way since then.

Bernadene Magnuson
Yeah. I just wondered too kind of comment on that too that [Indiscernible, Poor Audio.]
Okay. There we go. So is in terms of, you know, clients coming forward and wanting to pursue the approval or GRAS determination of a specific amount, and wanting, of course, in terms of consideration of the costs that are involved in all the studies. You know, being somewhat, I think, hesitant to start investing in any kinds of studies until they're very confident that that is going to add to the overall weight of evidence that they are working towards establishing.

So right now, I see, limited application of that at this point in time until we, we move forward, and so that's probably, you know, a consideration as well. Maybe we'll just -- oh, go ahead.

Sabine Francke
Just to follow up on the consumer confidence topic. Berna and I had been talking about this a lot. The consumer confidence drives some of this initiative. However, we cannot take it for granted either that whatever we might integrator propose substitutionally that that will be accepted. So if you look at the argument that has been made many times to say, if I don't have an exposure issue, we just may not have to care about it, but there may be people who still care no matter what the exposure, and I think we need to take that into consideration in our approaches.

I think we'll maybe just take a moment to look at some of the questions that are, that are popping up here too. One of them, I think, we've had addressed but maybe Nigel you can expand upon a little bit more is in terms of advantages, disadvantages of the NOAEL versus is the BMD and which is more cost effective.

Nigel J. Walker
Which is more cost effective? Well, I mean, in my earlier part of my career I did a lot of dose response model, and it's firmly grounded in the idea that it uses the whole dose response, it uses all the data. I mean, I'm a firm believer in the dose DMB, the NOAEL is predicated on the your dose spacing which can be manipulated and you can look at datasets where you go, ah, that's why they chose that dose because it allows, you know, you can actually use it to your advantage. Whereas BMDs is really based upon having, you know, all the data, but requires you to have decent dose space. Numbers of doses that's the only downside of the BMD, if you're doing dose response model on three doses in the control. You're certain your model fits tend to be a pretty, can be pretty poor if, depending on the shape of the dose response curve. So from a cost effective point of view, sometimes doing a NOAEL base is, you know, if you go classic guideline 3 or 4 doses and whereas the shape of the dose response curve is going to be important if you do the dose response model, so there are pros and cons to it.

Bernadene Magnuson
I agree. I think one of the problems, though when, you know from designing a study, and you don't know head of time exactly what that NOAEL's going to be. You're wanting to ensure that you have an adequate margin of error in terms of margin exposure between what you think are going to be the expected uses and what you're testing in your animal study, there's always that question, you know, in terms of whether or not you're going to miss it. Should you include an additional data point so that

increasing the likelihood that you establish a greater NOAEL, and I think, so it's a trade off, because as you said, you know, with using the BMD, you have that, you're not so dependent on exactly which doses that you chose for that study. And if you're, you know, considering the cost of an additional dose group or whether you're looking at perhaps enough to run an entire additional study, you know, that's where the cost questions come in.

Daniel Krewski
This is a good question that probably could warrant a whole colloquia in itself. There's a whole literature of strengths and weaknesses on various points of departure just 1 or 2 more that I can mention to throw into the discussion that you and Nigel were having. I think there was a paper by Dave Gaylor that looked at the actual risk of NOAEL. I think it was reproductive toxicity [Indiscernible] it's not necessarily a no risk level. The other one that I didn't get mention was the BMD level, the lower confidences level of the BMD actually gets higher has you increase your sample size so you're rewarding good experimentation, where I couldn't remember getting a high part of departure, whereas the NOAEL. The DRJ [Indiscernible] you're rewarding for bad experimentation in the wrong way, maybe I could just muddy the waters with another comment. We have introduced this concept of the signal to noise crossover dose, which is yet another point of departure we're still exploring its properties. If I take a long-term perspectives one the projects that we're look are working on in the [Indiscernible] center is what the time lag is between when a new idea is introduced in toxicological risk assessment until it become common practice I'll give you another example. We're looking at a whole bunch of these. [Indiscernible] on the benchmark dose a seminal paper in 1984, the first documentation I could find in our little study was in 1997, that's what 13 years later. So if we published our paper in 2011 on the signal to noise crossover dose, maybe by the turn of the next century it will receive some attention.

[Laughing]

Bernadene Magnuson
On the question of long-term, maybe we'll take another question from the, from the Internet members here. Where the question is, do high throughput tests and related bioactivity indications adequately reflect and pre-direct chronic toxic effect that only emerge after long-term low dose exposure so how do you address the duration of exposure effect? It appears that these predictive attest maybe he effect for only short-term a cumulative effects.

Nigel J. Walker
That's one the minefields that I'm not going to step into.

Bernadene Magnuson
That's one the minefields.

Nigel J. Walker
It's clearly the short-term *in vitro* tests are really the duration of exposure is shorter. I mean, that's no brainer. The question is, what are you actually measuring and is that biological pathway that you're looking at a measurement of something that could be. That you could look at accumulating hazards in a long-term model there's an all is PGS there that chronic exposures are due to an accumulation of insults over the course of a, of a two-year bio assay. You know we've done studies where we've done, you know start stop exposure, so in some cases yeah you require the whole time. In other cases you can clearly see that there are effects that it's only during a short window of exposure. It depends on the

mode of action for the particular carcinogen. Some of them are screaming [Indiscernible] you know, it's going to be different than something that requires persistent, and you know, injury.

Daniel Krewski
You're willing to give me a shot at that as well?

Bernadene Magnuson
Yeah.

Daniel Krewski
So this is a question that has come up many times over the last at the point years since the TT 21 C vision was put forward, and I can give a perspective on that. Nigel, I think said something similar to what I'm about to say is if we look at chronic toxicity and we understand the pathways by which those chronic effects occur, and if we can identify agents that cause the pathway perturbations that are along the route to long-term temporal effects and prevent those perturbations from occurring in the first place, we may have accomplished our job. Now there are some practical implications of that and understanding pathways and a sensitivity assays, but I do think we can hope that understanding the pathways by which chronic toxicity occurs we can target *in vitro* assays to try and focus on those pathways. I had another good idea on that one but it's escaped me for the moment.

Bernadene Magnuson
Another comment, yes go ahead?

Audience Member 6
In your GRAS you know of activities in the *in vitro* assays and exposure and saying well this is where there is a great margin between the two you have some assurance, but when there's going to be, one would assume there might be overlap at some point. Are we going to be able to develop guidance as to what the next steps would be to investigate this, I mean, I kind of think of it as a signal that somebody may be going wrong. I don't see us right now being able to just use that to say you can make a regulatory decision. You'd have to do further investigation. What would that be?

Daniel Krewski
I would have liked to have said something more on that, so I'm delighted to have this question, which allows me to say a little bit more. It's not my data, first of all. It's Barbara Wetmore should get all the credit in the world she has a series of paper that shows the same kind of pattern for the same sort of subset for all the Tox cast and the chemicals. In some of the GRAS and that type including the one I showed today there is an overlap between the upper exposure range and the lower level bioactivity and there you really will have to understand which biological effects are adverse in order to makes sensible use of those data. I was just blown away by the number of times in which the margin of exposure, at least for me, looks pretty darn comfortable so if I can set all those cases aside, I'd really reduce my workload to the ones where we do have the overlap between bioactivity and human exposure, but thank you for the question.

Bernadene Magnuson
Okay. We'll maybe take, consider one more question here. We still have a few minutes. There's a variety of interesting comments coming up here, but in terms of questions, one of the for regulatory purposes and risk assessment information and data from peer review publications is often used. It is often difficult to interpret these results as are not using standardized methods. This goes back also to not only sort of

predictive methods I think also we see that a lot in terms of animal testing. So the question is to what extent are researchers publishing in the academic literature using standardized testing and is it necessary to bridge that gap so that published data are more helpful for risk assessment?

Nigel J. Walker
I'm all over that one. Absolutely, yes, yes, yes, yes.

Bernadene Magnuson
I thought he'd like that question. So you wanted to read it.

Nigel J. Walker
I mean, it's, I mean, I mean, I eluded to it you know in my slides about the clarity of BPA, that's, the importance of that study is not about a BPA. It's about trying to begin to, how to bridge, you know, what we might call non-guideline study and investigative studies, be they done in industry, in academia, in Federal labs, and how do you start doing that? This is, this is actually something that's not peculiar to the US it's something happens in Europe, worldwide we want to see better use of information. And I think going back to Dr. Krewski you mentioned the systematic review approval, how do you gain confidence in that? You know exposure classification? Blinding of studies, independent databases. NIH is putting out that all data that is NIH funded, that should be transported in [Indiscernible] so people can do their independent analogous circumstances I think once we start doing that, there's going to be a higher burden in making sure what you put there and all the metadata that describe how you described it, how you captured the information that will allow a better independent assessment of the information and where the weaknesses are or where the strengths are. I mean, I'm a bit of a altruist on that one. If you build it they will come.

Audience Member 8
How much responsibility, though is relayed on these reviewers and the scientific journals that publish all this? They should say no periodically. And I don't know if it's happening. I mean, I realize the problem journalists have in publications, I mean, this, this creates this kind of problem. The data that was useless.

Bernadene Magnuson
I think that, I agree and I think up from my perspective also, I get so many questions from health professionals that are not necessarily toxicologists and they read a specific paper, which doesn't necessarily provide adequate information for the clear, you know, assessment as I was talking about, about whether or not, you know, is this really within the normal range? Was there, what other functions were assessed? What kind of pathology? I mean, it often can be very limited information that is published with a fairly, you know, strong conclusion often stated that undermines confidence in the safety of that ingredient, and it's very difficult without additional information to respond to that, and I think in my opinion, often that is somewhat undermines consumer confidences in the work that has been done. And you know, again, trying to enhance both what you've said in terms of, you know, trying to get additional peer review, I think is a challenge not only in our field, but in a lot, but hopefully, you know, sessions such as this are going to enhance understanding of some of the issues that we're trying to, to deal with. Yeah. Another question?

Audience Member 9
More a comment than a question, I'll try to make it short. This is follow-up [Indiscernible] question and Dr. [Indiscernible] answer to the question regarding using the new approaches of biological [Indiscernible] biological signals and to predict probability of toxicity effect. I'd like to think that it's

different from the regular way of thinking about the AP [Indiscernible] endpoint and look at the margin of safety when you look at the pathways, not one pathway definitely better than the other. And they're not born equally. You don't look at one assay or one signal. You look at the cluster of multiple signals and you put that together and you really need to incorporate a lot of, a lot of other information, the pathway and biological functions to incorporate that into the risk assessment, and that's different from the traditional way.

Bernadene Magnuson
Okay. We'll have a quick response, because I think we're about ready to wrap up so. So just a clarification on the upper panel, the green panel in those bio activity patterns, those were showing ranges across 30 or 40 I don't remember exactly how many, different *in vitro* assays so we're trying to look at multiple pathways, multiple endpoints in the same analysis, which is exactly your point. And Nigel, you may know how many different tests the National Chemical Genomic Center it runs through its robotic base test. It's actually a large number you can do simultaneously.

Nigel J. Walker
I don't know the number you can do it [Indiscernible, Poor Audio.] I mean, in terms of Tox 21, the 10-K we're about 100 plus assays but it's generally one assay per week for those, which is why actually in Phase III of Tox 21, we move into actually lower throughput but high dimensional assay using high genomics are where your actually assaying essentially all biological ways using, what's called the F1500 is what we're using for a moment.

Daniel Krewski
I know you're watching the time, but I want to say 1 or 2 sentences quickly on Nigel's point. A lot of discussion on Tox 21 has been a high throughput and [Indiscernible] testing if you look a high sweet. Medium throughput assay it's really a painfully of whole [Indiscernible].

Bernadene Magnuson
Okay. I think with that, I'd like to say thank you all very much for your attendance. I want to thank the organizing committee forgetting us going on this. Thanks FDA and SOT for their support for our participation and of course, both critically I want to thank all of the speakers and their excellent presentations, and, and participation.

We also would like to make sure you're invited to join the speakers for lunch if you want to continue the discussion, and we thank you for being here and everyone who watched on-line and brought in comment. Again it can't be done without the interaction. Thank you.

Thank you all.

[Applause]
[Event Concluded]