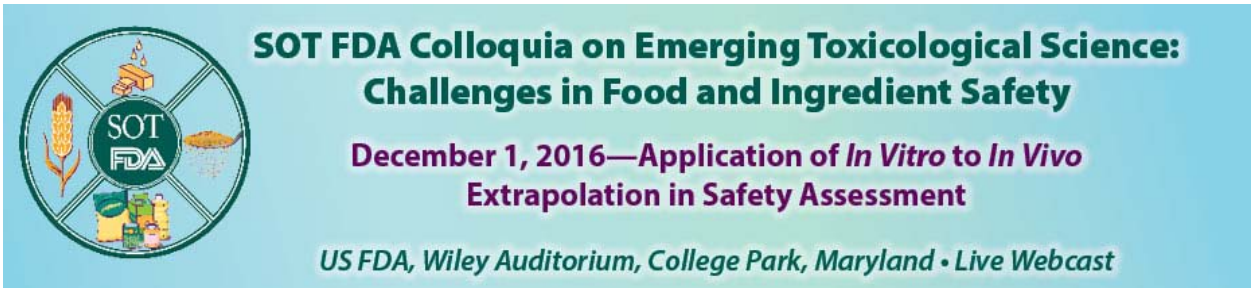

Real Time Captioning

Chair: Richard A. Becker, PhD, DABT, American Chemistry Council, Washington, DC
Co-chair: Lisa M. Sweeney, PhD, DABT, Naval Medical Research Unit Dayton, Dayton, OH

8:30 AM–8:35 AM	**US FDA Welcome and Overview** Mary D. Ditto, PhD, CFSAN, US FDA, College Park, MD
8:35 AM–8:50 AM	**Welcome from SOT and Introductions** Peter Goering, PhD, SOT Past President, US FDA, Silver Spring, MD **Speaker Introductions** Richard A. Becker, American Chemistry Council, Washington, DC
8:50 AM–9:30 AM	**Overview and Principles Underpinning *In Vitro* to *In Vivo* Extrapolation** Lisa M. Sweeney, Naval Medical Research Unit Dayton, Dayton, OH
9:30 AM–10:10 AM	**Data Requirements for Developing IVIVE Models** Nynke Kramer, Utrecht University, Netherlands
10:10 AM–10:30 AM	Break
10:30 AM–11:10 AM	**Examples Illustrating Potential Applications of IVIVE in Chemical Assessment** Miyoung Yoon, ScitoVation, Research Triangle Park, NC
11:10 AM–11:50 AM	**Opportunities and Challenges for Using IVIVE to Improve Decision Making** Weihsueh Chiu, Texas A&M University, College Station, TX
11:50 AM–12:50 PM	**Roundtable Discussion** Richard A. Becker, Moderator All speakers

The text below is the captioning of the live colloquium, not an official transcript.

8:30 AM–8:35 AM FDA Welcome and Overview-Mary D. Ditto, PhD, CFSAN, US FDA, College Park, MD

Good morning everyone. I'd like to welcome you to our series of SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food Ingredient Safety. Today's topic is the application of *In vitro* to *In vivo* Extrapolation and Safety Assessment. My name is Mary Ditto, I am the Director of Senior Science and Policy Staff in the Office of Food additive Safety here in the Center for Food Safety and Applied Nutrition. The primary role for the members of the staff, are to provide expert scientific, technical, and policy support for accomplishing the agency's goals of pre-market review and post-market assessment of food ingredients. They serve as a resource for OFAS, the center, and for the agency. The members of the senior science and policy staff have a very diverse expertise in areas including pathology, biotechnology, regulatory assessment, risk assessment, environmental science, informatics, and computational toxicology. Some of you may know that a member of the staff Allen Rudman has been instrumental in putting together and keeping the colloquia series going, so we certainly appreciate that. Here is some background on the colloquium series. The US FDA has partnered with the Society of Toxicology to provide this series of training sessions based on a memorandum of understanding and recognizing the organizations that share interest in scientific progress and the disciplines that directly and indirectly affect human and animal health and medicine. The memorandum outlines the opportunity for collaboration between the two organizations. In addition, to providing training events, workshops, and conferences to continuing engage the newest toxicology that is relevant to the FDA's mission and work.

This colloquium is intended to provide a forum to learn about the newest toxicology methodologies. It is to inform the work of FDA's [Indiscernible] employees by utilizing leading experts in toxicology from around the world. In addition, these colloquia are open to the other centers in FDA and the public. This offers an opportunity for everyone to hear the same information that the FDA receives. We are very happy also that we have great participation through the webinar.

These colloquia are not intended to be a discussion or make recommendations to the agency on a regulatory issues rather they are intended to discuss the latest toxicological and regulatory science with an emphasis on science. This is our third year and today will be the 10th colloquium in the series. This colloquium focuses as I said before, on the application of *In vitro* to *In vivo* in safety assessment and is co-chaired by Richard Becker, who is with the American Chemistry Council here in Washington and Lisa Sweeney who is at the Naval Medical Research Unit in Dayton Ohio. We will hear seminars from our 4 speakers, Lisa Sweeney, Miyoung Yoon from ScitoVation, is from Research Triangle Park in North Carolina, Weihsueh Chiu is from Texas A&M University in College Station, Texas. These talks will be followed by a roundtable discussion moderated by Richard Becker. If there is time at the end of the talks, you will have a chance to ask clarifying questions. At the end of the seminar, everyone in the auditorium can ask questions of the panel. For those of you online, feel free to submit your questions through the chat box. If you have questions for the panelists, you can put them forward at any time. We will try to get to as many questions as time allows.

In keeping with the theme of this colloquia of *In vitro* to *In vivo*, -- tomorrow Thomas Knudsen will be here to show us and to discuss EPA's computational modeling and simulation of developmental toxicity. You can see here an explanation of what this means based on high through- put screening and this will actually also be available via a webinar for those of you who can online, you can take a screenshot of this or if you want you can contact us and we will send you the information.

A few administrative things for those of you here in the auditorium. Basic information -- the restrooms are just outside the auditorium half way down the hall. There is a public cafeteria just outside the Wiley Building on your left, which is available if you need some food and finally lunch with our speakers will be here in the Wiley building in room 2A023. Now it is my pleasure to introduce Dr. Peter Goering, the past president of the Society of Toxicology and the supervisor research toxicologist that FDA and the Center for devices and radiological [Indiscernible].

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

Thank you, Mary. On behalf of the Society of Toxicology, I want to welcome everyone in the auditorium. We have about 80 that pre-registered. We have almost 600 people that pre-registered for the WebEx. This is a wildly popular topic as evidenced by the registration, so welcome to all of those in the room and to all of those checking in online.

About six years ago, the Society of Toxicology was engaged in developing one of its' strategic plans for the next 5 years. We decided that it was a priority to establish relationships with a number of Federal agencies to work on common goals. Some of these goals included using the best science for regulatory decision-making as well as engaging in training on staff on the state-of-the-art concepts. This was the result of a memorandum of understanding that was established with the FDA and the Society. It has been a very successful relationship and collaboration. I'm pleased to announce that this Monday on December 5th, the FDA Chief Scientist Luciana Borio and SOT President, John Morris, will sign another memorandum of understanding for the next 5 years. We hope that will be an impetus for us to continue these colloquia which have been very successful.

Over the past 2 years, the FDA and SOT have collaborated on nine previous colloquia and the topics are listed here. These have involved training in understanding and state of the art concepts in risk assessment. This is our 10th today as Mary said, so we are very pleased about this ongoing series. There is an opportunity for the staff here to learn from a panel of experts across the globe in state of the art concepts. Not only for those of us who are toxicologists, but we have online graduate students and postdoctoral fellows from across the country, and from around the world, who are listening in. It also serves to help train our future toxicologists.

Some feedback from the first colloquia is that they have been very successful in terms of the quality and the kinds of content. The data is from our survey that we give at every colloquium. I encourage your office to go online to fill out the survey. It tells about how we are doing. We also would like to have topics suggested to us that will be useful in your future colloquia. These colloquia would not be successful without a stellar organizing committee. This committee is made up of representatives from the FDA as well as the Society of Toxicology. Those from FDA, I think they are all from CFSAN are Jason Aungst , Suzy Fitzpatrick, Alan Rudman, especially thanks to Alan for taking the lead on these colloquia and for the work that you do to make it successful. Jeff Yourick from CFSAN those from FDA include our committee chair Ivan Rusyn from Texas A&M. He has been a driving force in developing these topics in these colloquia. It is my pleasure to introduce Richard Becker. He is the chair of the colloquia today and Lisa Sweeney is co-chair, today she is also one of the speakers.

Let me introduce Richard Becker. He has been a senior toxicologist at the American Chemistry Council for 16-17 years. He leads the Council of Science and Research division. He directs the

long-range research initiative. This is a research program that is designed to modernize and improve chemical safety assessments. He stands among giants in risk assessment. He has just last year received the Arnold Lehman award from the Society of Toxicology in recognition of his contributions to the field of risk assessment. There are some giants that have received that award in the past, so he stands in good company. That said, before I asked Richard to come forward, there was a mistake in one of the slides that Mary presented about the EPA seminar tomorrow by Tom Knudsen. It will be held from 10 AM until 2 PM Eastern time, please note that correction. Richard, I would like for you to come up now and start the colloquium. Welcome.

8:35 AM–8:50 AM Speaker Introductions--Richard A. Becker, American Chemistry Council, Washington, DC

Thank you very much Peter. Really appreciate it. I'm humbled by your description of standing with the giants. I think I'm standing on the shoulders of giants always, so I appreciate that. Also, I really want to thank the organizing committee for having the foresight to pull into the colloquia series the session on *In vitro* to *In vivo* Extrapolation. The sciences are challenging for us who are not from [Indiscernible] but this is an evolving field and very important for all of us who are interested in improving risk assessment to understand pay attention to and better understand the strength application [Indiscernible] and challenges we face in, going forward. So is really a pleasure today. My job today really is to keep us on time and to help the audience both on the web and also here in person engage with the speakers.

We have a number of speakers today to cover the range of challenges and opportunities in the *In vitro* to *In vivo* Extrapolation. We will start with Dr. Lisa Sweeney to present an “Overview and principles underpinning *In vitro* to *In vivo* Extrapolation.” Then “Data Requirements for Developing Models” by Nynke Kramer. Miyoung Yoon will speak about the “Examples Illustrating Potential Applications of IVIVE on Chemical assessment.” Then Dr. Weihsueh Chiu will talk about the “Opportunities and Challenges to Improve Decision Making”. Just a reminder on really focusing on the sciences that Mary said here, not regulatory decision-making per se but this is a new field that is evolving. As it evolves, we will be looking at how to incorporate the science bases.

It’s all about the science today. Try to remember that when we are formulating questions and reminder to those who were on the web, this is an opportunity for you to engage as well, so please do in the chat area sending questions. Our schedule is a bit tight today so we may not have a chance to get to each question after each speaker so hold on to those a little bit, both in the audience because we will get to that in the Roundtable discussion. So, that's it in terms of ground rules.

I would like to then introduce our first speaker Dr. Lisa Sweeney. Dr. Sweeney has a broad range of experience in the application of toxicology chemistry and engineering principles [Indiscernible] environmental sciences. She has over 20 years of experience in risk assessments from biochemical engineering. She is author of peer review publications. She has really focused her research on developing IVIVE models and application of risk assessment and experimental design. She received her education at [Indiscernible] University and PhD at Cornell in chemical engineering and completed postdoctoral training in modeling at [Indiscernible] and American Board of Toxicology and a certified hazardous materials manager. Dr. Sweeney.

8:50 AM–9:30 AM Overview and Principles Underpinning *In Vitro* to *In Vivo* Extrapolation-Lisa M. Sweeney, Naval Medical Research Unit Dayton, Dayton, OH

Thank you Richard and thank you to the organizing committee for inviting me here today. I hope that my talk will help you to better understand the talks that follow because they are the real experts on IVIVE. My background is more general in physiologically based -- so they don't have to give all the background and hopefully this will equip you to understand the additional talks that follow. I can disclose to you is that I have no conflicts of interest and the opinions express here are mine, and not those of the Navy.

In case you signed up for this [event] because these colloquia have been a great series and you have forgotten exactly what this one is about, I just wanted to remind you of the general things about this topic and some of the rationale that the organizers put in their promotional materials. We're here today to help equip the disciplines to understand how to consider what do all the new *in vitro* data mean because this is a growing area of assessment for various types of chemical agents [Indiscernible] and industrial chemicals. The question is how can we better understand what it means when we have a concentration that does something *in vitro.* I think of toxicology is half of a football score. If you tell me my score -- my team scored 13 points it doesn't really tell you whether they won or they lost. It depends on what the other side did. If they scored zero I would have been sure they would've lost and if they scored 50 or 60 that almost certainly would have won. Thirteen, that is kind of a borderline area and unfortunately they may have lost, but maybe if they scored 13 points tonight it will be enough. You have to put toxicology in the context of exposure assessment. So physiologically-based pharmacokinetic modeling is one way to basically backup from an internal concentration and say what does that mean to an exposure. Exposure is usually something that is a little bit easier for people who are not pharmacokinetisists to understand, It is not good to be exposed to a certain amount because this is bad stuff. So it does provide context to the *in vitro* data these generate more and more to understand the hazards of chemical agents.

I don't know if the organizers actually said this sounds like a good idea or whether they actually did say this is a growing area and an important area, but one way I look at some of the trends is a look at how many pub med hits a certain topic has over the years. And most important would be the top line which is toxicology and *in vitro* and if you look at the 80's and 90's into the early 2000's it was fairly steady but it has been growing in recent years and this is a logarithmic scale so it is definitely growth. It doesn't look like it's going at a high slope but if you are trying to keep up with the literature when it goes by a factor of 2, that is an issue for scientists. And if you look at the next row of symbols that is for basically physiologically-based pharmacokinetic modeling which has a certain background level, also has been increasing over time. If you look at the bottom row, you can see that in the early years of pharmacokinetic modeling there were not a lot of these studies that incorporated explicitly a lot about *in vitro* enough to make the abstract in a pub med entry but that has also been growing steadily in recent years.

If you look at risk assessment, *in vitro* kind of jumps around a little bit in the earlier years because I think these terms were not in focus much and in this case I looked at *in vitro* as a fraction of the amount of hits on risk assessment. Risk assessment is a very general term to include things that have nothing to do with toxicology. That you can see in the last couple of years has been an upturn in the number of hits that you get for *in vitro* and risk assessment. So this is definitely a growing thing and if you are working in the field, you will be that much more recent that you will need to understand the topics in order to types of data. That you will see or perhaps you are generating the sort of data and to understand that there being used more and risk assessment gives you a context of what you are doing, and why, and who is using it.

I will start very briefly with some principles of toxicology and discuss some of the how, what, and why of physiologically based pharmacokinetic modeling. A large portion of the top will be how to evaluate models for use in risk-based decision-making and we will talk a little bit about IVIVE and some of the principles that underlie the application of IVIVE in safety and risk assessment.

If you go to the root of the word toxicology, it goes back to the Greeks. It comes from words that mean to study poison. That is a little bit specific then actually how we use it today. Usually today, toxicology is the study of adverse effects of chemical, physical, or biological agents on both individuals, animals, or populations. Given toxicology studies, the objective is to assess the nature and probability of the effects on human health or animals and the environment from exposure to toxic agents. As I mentioned, you combined toxicology with exposure assessment to provide a basis for risk-based decision-making. That is how it is used when I explain to people what I do. I usually explain how someone else uses it. Some of the faces go away. Then I explain how it is used.

The first law of toxicology is very relevant to *In vitro* to *In vivo* Extrapolation. The dose makes the poison. From an IVIVE perspective it is not just the dose that makes the poison it's the target tissue that makes the poison. Toxicology dissolves somewhat of a black box that you can go from a dose to a response and you don't necessarily think a lot about everything in between. From toxicokinetic perspective opens up the white box you can consider the exposure in terms of an internal dose or perhaps a target tissue dose. Basically, the more you know about the dose, hopefully gives you a better ability to refine the dose response relationship. It was just at the heart of the first law of toxicology a low does is not going to do something, where they were not going to do something in the higher dose. It was going to do what it does with higher frequency at a higher severity.

We will focus more on the internal dose imagery. We have talked specifically about physiologically-based pharmacokinetic models and the question is how they are they related to risk assessment. Because chemical risk is intrinsically about the dose, the PBPK modeling is a tool that describes the relationship between exposure and internal dose. The better the estimate of dose, the better the character ration of the dose response and physiologically-based pharmacokinetic modeling can be used to inform the uncertainty of other extrapolation such as inner species extrapolation and extrapolation from general populations to more susceptible up populations. So, physiologically-based pharmacokinetic modeling in particular PBPK modeling involves understanding of biology, chemistry, and mathematics. Examples of some of the biology includes basic things like tissue volumes, blood flow rates breathing rates. Some of the chemistry involved is solubility of an agent in a particular tissue that is related to the tissue composition. For example; how much fat, how much protein, how much water.

When considering a physiologically-based pharmacokinetic model you need to consider things such as [Indiscernible] turned into something else. How that is excreted from the special tissue functions in particular of some of the portals of entry such as the lung or the gastrointestinal tract. PBPK models are simplified descriptions of reality and depending on how much information you have on the chemical and how much -- what your inclined to consider for a particular application you might have varying numbers of compartments that you would describe and each of those are described by a mass balanced equation. In particular, there are different types of doses that can be computed with the model such as a [Indiscernible] concentration or amount metabolized so are things that can be computed with the model that can't necessarily be measured directly in an *in vivo* situation. In developing a specific model, you might consider what data is available. For example, if you have target tissue measurements you might include

those in the model. If you have biomonitoring data such as saliva, you might actually have a saliva compartment. But you're not going to see that in models if you don't have that particular tissue, because it is such a small component.

I've included a few example equations to give you a bit of flavor what the model actually looks like when you get down to the bare bones. These require certain assumptions and these will not appear in every model. For example, in a fairly simple description of gas exchange, you will have an assumption of equilibrium between the in health concentration plus the venous blood and an equilibrium. You will also have a certain concentration that is exhaled and goes in the venous blood. You could describe the respiratory tract as several different compartments because obviously you have the nose and the lungs. But this is an example of a simplified expression that is often adequate. The venous blood equation which follows is basically [Indiscernible] you have a certain amount that comes in and a certain amount has to come back out. You can't just have the typical disappearing. Tough to go from one will plug into another. The outfielder air -- [Indiscernible] air in the air concentration which is based on the thermodynamic properties of the chemical.

For metabolism, it can be described as -- with varying complexity depending on how much information you have. In this case, it is written as a [Indiscernible] equation with a single [Indiscernible] constant. If you know that there are multiple pathways, you can have a much more complicated expression of the metabolism. In the tissue, you may or may not have metabolism but you have the flow; blood flow from the arterial supply. You will have a certain amount leaving. You might have some that is metabolized and otherwise it accumulates in the tissue and the assumption that the venous blood is in equilibrium with the tissue when it leaves. These are assumptions that may not hold in every situation.

There are many ways that PBPK modeling can be applied in risk assessment. One approach is when you do not have a separate exposure assessment, is to use reverse dosimetry to estimate what a past exposure was. In this sort of simulation you would have a known amount for example in the blood or urine and you would use a pharmacokinetic model to detect the exposure and you could compare that directly to concentrations that are known to have an effect and a well-controlled study or perhaps in an animal study. And dose response modeling, PBPK modeling is a powerful tool because it can be used to combine data within various study designs. For example, if a chemical has been studied by both oral and inhalation routes you may assume that the target tissue toxicity is related to concentration in a given tissue. Then you can predict it from both types of models. In that way, you can combine the results in different studies in order to have additional data to do the dose response relationship on combined information which led to internal dose instead of having to analyze the studies separately. [Indiscernible] extrapolation similar to the idea of combining studies for different routes. Studies conducted by one group but not by the other. For example, if you have an oral study but you are interested in inhalation toxicity, you can use pharmacokinetic modeling to make predictions of what a held concentration would produce the same effect as occurred in the oral study. In that way, you don't have to utilize limited resources to basically repeat the same type of study. [Indiscernible] When you study about one root, why would you want to do another study if you can have a reliable extrapolation based on modeling?

Also inter-species extrapolation is common use of pharmacokinetic modeling especially in situations where you cannot do human testing. In particular, with inter-species variability, you may have data on, for example, a healthy population but you may wonder how a more susceptible population would be affected. For example, if you have a drug and you test it in healthy people, but then how does the different health status affect someone else -- somebody

that has for example kidney problems, and cannot as effectively eliminate the compound. How much greater or lesser risk to them it will have will depend on various aspects of health status.

A particular challenge is reproductive and developmental toxicity when considering pharmacokinetic modeling because the physiology in the anatomy are changing very much throughout the duration of the study. For example, in a two generation study, your offspring may be exposed via the placenta when they are developing; when they received chemicals through lactation, or when they are nursing pups and then they go on to become adults and they have their own direct administration so they are changing the mode of delivery; the route the life stage. Pharmacokinetic modeling can help evaluate the dosimetry and the various life stages of a given single study.

Also with reproductive and development toxicity, you have a large number of endpoints. You may have different windows of susceptibilities of the ability to calculate internal dose in these different stages, it can give you more options for interpreting the data.

To give some more specific examples, I have some examples from a study of ethylbenzene that I was involved in several years ago. It was for EPA's voluntary children's chemical evaluation program and it was reviewed by a peer consultation panel. Though the resulting paper is over 60 pages, if you really wanted all the details you would have to go back to the appendices of the report, because it was such a large evaluation. Pharmacokinetic modeling was used in a lot of ways. [Indiscernible] used for exposure assessment and that a hypothetical workplace scenario was used to estimate how much ethylbenzene would be transferred to a nursing infant who's mother worked in a workplace that had ethylbenzene. It was also used as a reality check on biomonitoring studies to see whether the source of ambient concentrations of ethylbenzene were consistent with the blood concentrations that were measured in things such as the [Indiscernible] study. It is used as an experimental design in that it was the two generation study that was conducted primarily by the inhalation route. But, it is not appropriate to take the mother rat away from the newborn offspring for a while. During that period the animals were dosed by [Indiscernible]. Pharmacokinetic models were used to determine what will be an appropriate dose that would be consistent with the inhalation part of the study as well. We also use pharmacokinetic models in developing cancer and non-cancer reference values that were based on internal dosimetry. This included using [Indiscernible] extrapolation because there were chronic studies by inhalation and only sub-chronic studies in the oral study. We wanted to compare whether we should use oral studies or whether we should do inhalation studies. In addition, we wanted to compare departure for the oral chronic reference values and also inner species extrapolation because there's not a lot of human data on ethylbenzene exposure.

The pharmacokinetic models that we used, included a mouse model that was developed specifically for this program and therefore, it was used in the assessment exactly, is published. The rat and human models that already existed were not tailored specifically for this program so there was a need to expand them. Modify them a little bit, because the models were based on inhalation. There was consideration of the oral route so there was limited data for the oral dosing. We were able to parameterize the model for oral dosing. We also modified the description of metabolism to better describe higher concentrations to have two pathways of metabolism. For the human model, it was not originally developed as a lactation model. In order to do the infant exposure assessment, we needed to add a mammary compartment to the model.

Also the model for humans originally did not have any long metabolism because lung cancer was identified in the rodent studies. They developed *in vitro* estimates of long metabolism for

mouse, rat, and human and used those estimates. We added those to the human model so that we could do inner species extrapolation to do a human risk assessment for lung cancer.

As part of this, there were some “what-if’s” that were done because there was no detectable metabolism of ethylbenzene in the human lung tissue. What if the human lung metabolism really was as much as in the rat? That will be basically an upper bound condition so we looked at the simulation and if we put a bunch of metabolism in the lung; similar to the rat. How does that affect the models in the upper line is the original model in the lower figure line? These simulations indicate what the ad of metabolism and it is somewhat in the range. But you can under predict the measured concentration in alveolar air and in blood, so it is definitely a conservative assumption to assume that the humans metabolize as much. And so are sent over band on the cancer risk that by using the rat *in vitro* rate that the assessment will be conservative and therefore protective of human health based on the amounts of metabolites that will be expected to be generated in human lung.

Having given a specific example, I would like to [Indiscernible] evaluate a particular model that might be used in risk assessment. The goal of a model evaluation is to assess model confidence is it for a specific application or wondering how can you use it because there are various ways of using them, to be considered key are different applications. For example, using a model for prioritization of different chemicals or driving a slope factor for this has different expectations. [Indiscernible] those different types of applications that might constrain what you think is an acceptable margin of exposure between a level that you think might present a risk and what you would prefer, because obviously you want to minimize risk. Assumed that the model has already been built, but in reality, model building is iterative, as I've mentioned in the ethylbenzene example. An initial model evaluation may suggest modifications, or further developments that are required in order to be adequate for a particular purpose and this is done through a series of questions that are adapted from a publication by McClanahan et al. Question is, how biologically realistic is the model versus how realistic does it need to be? The model that I showed initially had several different compartments. You may not really need that many depending on the properties of the chemical and how many compartments you have data about anyway; so lumping versus putting is always an issue in modeling. The question of model suitability includes what species has the model been developed for. If you are looking for a PBPK model for marmot, I've never seen one, but it doesn't mean it cannot be created. [Indiscernible] exposures inerrant something that could be simulated with the model needing to add oral exposure to the ethylbenzene model.

Simplified study state models may be long-term exposures that may go on to be fairly suitable over time, but it might not always be suitable for a short dynamic life stage such as pregnancy. And again, that's going to be dependent on things such as the chemical properties. Something lightly at that accumulates over time is not going to be very amiable to a steady-state model. Are the mathematical descriptions and computational implementation adequately verifiable? Are you going to use the results of someone else published or do you actually get to see the equations and codes so that you can check for inconsistencies and errors because they do creep in. I find it very challenging to reconstruct model strictly from a literature description. It is almost always frustrating and so being in direct communication with the person doing the modeling is important.

Another question is the model verifiable? Can the previous simulations be reproduced? Sometimes it is something as simple as when you have study specific value such as body weight those might be used for a particular simulation but perhaps you only have a general table the only has default values and sometimes is hard to re-create a simulation because small

details have not been adequately documented. When people ask whether a model is good usually what they mean is how accurately does it predict the data. They are not used asking about the appropriate model structure. The question of how good is good enough -- a lot of models that you will see published often have been tested only against in-house data. Sometimes there is not really a comprehensive evaluation of the model against all of the available data and this is particular the problem with industrial chemicals, as was something as specific as a pharmaceutical that is under development. It widely varies. Some people are more thorough than others.

The question of how good is good enough? There is some sort of outside guidance which says that on average model should be within a factor of 2% should be used in risk assessment and I would take this to mean a more formal risk assessment such as driving a toxicity reference value as opposed to a prioritization activity but that is something that is evolving I would say.

Of course how well known or expected -- how well the model is expected to perform in the scenario of interest -- for example if you have been if the model has been dramatized that higher concentrations it's easier to have [Indiscernible] than the other way around. If you go from low to hot you're more likely to go from first metabolism to a [Indiscernible] metabolism. The comparison of how the model is developed versus how it is being used is also a consideration in evaluating a model. One thing that can be challenging is to evaluate the parameter values because there are often so many that is hard to give a lot of attention to every single parameter in the model. One question is whether the values are consistent with well-vetted collections in particular for the anatomical and physiological parameters although you could have as I mentioned for some of the intra species variations, you might have less data on physiology associated to specific health conditions but these are increasingly available because this is an application of great interest. And in terms of the parameters, has the variability or the uncertainty of the parameters been characterized? Sometimes in and optimize model you just get an optimized parameter value without any sort of plus or minus say you're not sure how precise that estimate is.

In a lot of cases, the parameters are not directly measured and so the question is how supportable some of the parameter values are, for example is pretty standard to use partition coefficients that are the same across species and there is a fair amount of data to indicate that this is not something that is highly variable across species. So generally, that is not a concern if you are using a rat partition coefficient in the human model. Another approach that could be used to support parameters is known as the parallelogram approach. For example, when *in vitro* values are developed for the rat and can be compared to an *in vivo* model that suggests that use the same approach to measure the *in vitro* parameters in the human. And then through the results for the human even though you don't have human data to compare to, that supports the reliability of the human parameter values. Other examples are read across, for example if you have values for methyl, ethyl, and propyl, but not for butyl, you can sometimes interpolate throughout that series to have a good guess especially if you are between parameters instead of extrapolating the on to [Indiscernible] and that is another [Indiscernible] parameter evaluation. If you are using a model that is purely predictive and you have optimize parameters, one thing is whether the type of data that you have our suitable for identifying the parameters. In other words, is the output actually sensitive to the parameter that is being optimized? Therefore, the model competence may be enhanced are limited based on the answers to this type of questions and may have implications for how you can use the model.

I wanted to talk more about sensitivity analysis that involves a determination of how to change input effect affects the model output and there are various approaches. You need to clearly

define what your referring to because the sensitivity can be dependent on for example the blood concentration they not be sensitive to the same parameters as the amount metabolized. And the sensitivity during exposure may be different from the sensitivity after exposure for example in an inhaled compound.

One way of doing a sensitivity analysis to compare two groups. You may compare healthy adults to adults with a particular condition such as renal failure or people exposed for two years versus 20 years. That is a form of sensitivity analysis. More commonly used sensitivity analysis is a local sensitivity analysis such as when you make a 1% change in input parameter how does that change the output. It is usually normalized so that for example a percentage change in the output to the percentage change in input and if you have a direct linear relationship you increase the dose by 10% and you increase the blood concentration by 10% would have a normalized sensitivity coefficient of 1 for example.

Having a sensitivity analysis can help prioritize parameters for uncertainty or variability analysis. For example in the Monte Carlo simulation you might very the parameters based on the knowledge of how those parameters vary in a given population and then have an overall result for you can compare the median individual to a certain percentile. By using a sensitivity analysis you may limit the number of parameters that you introduce into your Monte Carlo simulation for your convenience. And if you're willing to assume a normal distribution or have evidence that parameters are normally distributed you can actually get to the preliminary prediction of variability output based on the variability of each individual parameter and the sensitivity of those parameters.

By using sensitivity analysis you can then see which parameters are going to be the largest contributors to model variability over all and of that way you can focus on a smaller number of parameters then all of the parameters that are in the model. Particularly with respect to IVIVE applications the *in vitro* parameters may not actually in up into my sensitive parameters or when they are you know that you need to focus on reliability of the methods for which these parameters were derived. So, the purpose of toxicology is risk assessment. And how does IVIVE fit into that? Those that were involved in the Tox21 efforts recognize the need for context for *in vitro* effective doses that the robots were going to help undetermined from all these different studies in different endpoints and different cell types. They found that there was not a lot of traditional *in vivo* toxicology data that were available for the chemicals that were in the test program. You can see from this figure that really they were not going to be up to provide much context for the *in vitro* effective concentrations based on *in vivo* data that were already available so they looked to *in vitro* methods to more rapidly expand the amount and type of PK data to get have in order to divide context to all of the chemicals in this program.

I would like to mention that IVIVE is not new. If you go back to a 1982 abstract you can see that they are using *in vitro* experiments with liver microsomes to determine Km and Vmax by looking at the plasma binding and looking at penetration rates based on lipid solubility. Unfortunately in this example it didn't agree to well the *in vivo* data but they found a particular group of parameters that turned out not to be well predicted by *in vivo* and they only needed to adjust that particular set of parameters in order to get fairly good agreement so this is from 1982. Like I said, IVIVE is not brand-new but there is more of a demand for because there is more *in vivo* data that we need to provide a context for. Partition coefficients for volatile components based on a headspace approach were have been retooling determined to through *in vitro* measurement since the late 80s a compilation paper was published and they had already been accumulating the values for several years by then.

The *in vivo* metabolism has been predicted from *in vivo* metabolism by skilling up various approaches to measuring it either with directly with the [Indiscernible] or with subcellular fractions such as microsomes and cytosol and the cells would be scaled to the number of cells in a particular program weight for example in liver or another applicable tissue. For subcellular fractions you need a yield of how much cytosol or my present you get program of tissue. Another approaches to used cDNA, expressed enzymes, and scale based on content in particular tissues which make vary with demographic characterizations. So, basically their building on approaches that have been used for 20 years but they are trying to do this in a context of higher through-put toxicokinetics thats faster than an *in vivo* study. Some of the simple models that have been described and from the more recent literature of based on human data -- human *in vitro* data for example with mixed donor [Indiscernible] from a 2 donor pool and purging bindings from a six donor pool and from these *in vitro* clearances they are able to estimate metabolic clearance and then estimate the free fraction that can then be clear through [Indiscernible] filtration and produce an estimate of a steady-state concentration relationship between the [Indiscernible] concentration and the dose rate that is based on these *in vitro* estimated clearances.

To pull us into a summary of a few limited numbers of principles, the basic first law of toxicology is an important principle in IVIVE is that the internal dose makes the poison. The second principle is that physiologically-based pharmacokinetic models are particularly well-suited to predict internal dosimetry for countless hypothetical scenario so that you can simulate things that you either cannot should not or don't want to test because it is too much of a hassle. The third principle is that appropriate structure and parameterization are essential characteristics for any predictive model but to assess whether an IVIVE model is fit for purpose there is a requirement to understand both the assessments needs and the models limitations there is no one-size-fits-all. An important principle for going forward is the new higher throughput more physiologically realistic *in vitro* assays should be yielding parameter estimates that better reflect *in vivo* disposition and therefore we expect better model productivity and we had act in 1982 when some of those early *in vitro* to *in vivo* extrapolations were done.

Some other resources that might be of interest, EPA workshop and I think we have limited time for questions if you have any specific to this presentation the free to ask a couple now. Our moderators will determine how many we can answer but otherwise questions will be very much appreciated in the roundtable. Thank you for your attention.

[Applause]

Richard A. Becker
We have time for one question. One clarifying question perhaps? Okay. Well thank you this way. If you have any questions on her presentation or think of any later, please hold those for the Roundtable discussion. Thank you.

[Applause]

While we're pulling up our next speakers slides let me introduce our next speaker. We're very pleased today to have Dr. Nynke Kramer joining the speakers. She teaches toxicokinetic, pharmacokinetic, toxicological risk assessment, and quantitative *In vitro* to *In vivo* extrapolation . Her research focuses on developing techniques to models to improve extrapolation [Indiscernible] concentrations [Indiscernible] doses relative to humans and animals. She was awarded the [Indiscernible] for research and received her -- graduate degree from the

[Indiscernible] University of Oxford. Dr. Kramer will focus on more of the data requirements that are needed for developing IVIVE models.

9:30 AM–10:10 AM Data Requirements for Developing IVIVE Models--Nynke Kramer, Utrecht University, Netherlands

Thank you for that introduction. As mentioned I will talk about the requirements for developing IVIVE models and this is part of our research that we do it the Institute of Risk Assessment Sciences and we try to approve the productivity of *in vitro* tools for estimating the parameter in *in vitro* to *in vivo* models.

I will start with stating that I have no conflict of interest and the views that I express are my own and not that of my Institute necessarily. I do hope they with me, of course. As part of the topic I will be talking about, I'll try to answer the central question of what data is needed for IVIVE models. Start by introducing how we use *in vitro* assays in risk assessment and how we hope to use them in the future. How we hope to use them in the future will be in the paradigm of *in vitro* to *in vivo* extrapolation and integrated approach for we combine several *in vitro* tools and [Indiscernible] tools to come up with a human equivalent dose that we expect to be toxic, given *in vitro* data. I want to talk about the parameters that are required for IVIVE model specifically been a PBPK model. I will discuss some of the assays that are available in both toxicodynamic assays, as well as toxicokinetic assays I want to go into more detail on some of the challenges of translating *in vitro* effects concentration to that of *in vivo* dose.

Introducing for those who do not know, there are two types of *in vitro* assays that we could do for these IVIVE models and these include toxicodynamic assays and toxicokinetic assays. To discern a dose response curve or effect concentration--excretion in the body tend to look like figure that I put up where you put your compound and one in you observe the rate of depletion with the passage of across to the other end.

These assays would both be needed in [Indiscernible] testing paradigm that has been mentioned in the national research Council in that report Toxicity Testing: A Vision and a Strategy. These *in vitro* assays are no longer to be used simply as screening methods but as a hazard identification and characterization methods so that's really to express the effect concentration to which we assume adverse outcome. This is where we need these IVIVE models because we estimate chemical exposures producing a target tissue exposure in humans which is equivalent to those associated with the effects in *in vitro* test system.

To illustrate this I put up a model by Louisse and colleagues back in 2010 which illustrates the type of information that you would need from these *in vitro* assays to come up for the human equivalent dose. What they did here is they exposed mouse embryonic stem cell tests to the metabolites of [Indiscernible] and expressed these in concentration effects curves the effect concentration appear -- up here is equal to the concentration in fecal blood and using PBPK model they extrapolated of what would be the predicted oral [Indiscernible] toxic dose present of glycolethers . In order to understand the rate at which these are metabolized -- the use *in vitro* toxicokinetic assays in this case human as well as rats. When they express the rate of metabolite formation and from there you can see that they did a pretty good job at estimating the dose, the embryotoxic dose that you would need to give a parent rat [Indiscernible] fetus.

This model also shows that it is an integrated approach. We start with the PBPK model and the compartments the we include will depend on the exposure route that was interested in and we can also use the sensitivity analysis to see what parameters requires the most precision since it

has the greatest effect on the outcome. And from there we can then decide what *in silico* code tools are what *in vitro* tools we can use to estimate the parameters and there is no clear guidance as to which one to use yet. But it is important for *in vitro* [Indiscernible] is an assay that measures and has a readout that we know is related to an adverse outcome in a human. For example in this is not well developed that we are working on it will we are talking about adverse outcome pathways were really need to understand every step for make molecular [Indiscernible] how that translates into an adverse outcome. Of course, the level of precision that we would need for these parameters will depend on the model and questions that we ask in the margin of exposure the outcome has compared to exposure that we expect and that has been said before it is a tiered or iterative approach. This is nicely illustrated here with a picture from Miyoung Yoon, which illustrates at what point do we used a different tools to dramatize these PBPK models. In the first approach with use [Indiscernible] for quantitive structure relationships and property relationships to both estimate, for example the metabolites that we can expect or the types of organs that we expect toxicity to happen so we can include these in the PBPK model. We can then verify the *in vitro* kinetics tools where we can estimate for example the intestinal uptake and metabolism of a compound the renal clearance and partitioning to the tissues as well as the binding to [Indiscernible] protein which we could then also feed our parameter estimates in the PBPK model. Of course we needed a dose response [Indiscernible] or concentrates the effect curve [Indiscernible] assays to calculate an effective dose which is down here.

To understand what parameters are required to be parameterized or required data of put up a generalized PBPK model that includes some of the parameters you would normally find in these model. So the amount of a chemical toxicity in a specific tissue will be dependent on blood for rate for example and the partition coefficient to the tissue and the first type of parameters that are in these models are is illogical or anatomical parameters these include tissue blood for rates and respiration rates, tissue volumes and [Indiscernible] filtration rates and these are will characterizing you can find some of these parameters and others in the paper [Indiscernible] 1997 and the nice thing here is a lot of the variability across [Indiscernible] parameters is also [Indiscernible] and you can include that into your model. Less simple are the physical chemical parameters in these PBPK models. These include tissue blood to partition [Indiscernible] require tran21 [Indiscernible] concentration of tissue versus the concentration that is available outside in the blood.

Protein binding constants are important because it is the unbound fraction[Indiscernible] uptake into a tissue and tissue clears elimination rates also parameters in the PBPK models for IVIVE and they can include [Indiscernible] very simply a clearance which is just a ratio of [Indiscernible] and of course that can include the viability of the compound. There are a number of *in silico* tools available and I would just explain what they can do. You have many [Indiscernible] the estimate partitioning so tissue partition constants as well as protein binding constants in the challenge really is for these *in silico* models is to have determine metabolic clearance parameters in these models. [Indiscernible] That we do have are mostly quantitative and can for example estimate the type of metabolite we could expect to be formed and these [Indiscernible] toolbox and multi-case but for these clears assays we generally use *in vitro* tools.

To start with developing such a IVIVE model we use a tiered approach that we start simple and we use [Indiscernible] parameters that have uncertainty the give you an indication of the range in which you would find an outcome or does in humans. The first tier of model development will be passive diffusion and we assume that the chemical is passively diffused into tissues [Indiscernible] what we generally need is a flux [Indiscernible] which we can obtain from the permeability constant and *in silico* and multiply that by the service area in the concentration. So

I've got this picture up here. These are the parameters that you would include in this model for absorption and of course include parameters of distribution. Here we also assume that diffusion -- the uptake into a tissue is diffusion -- dictated by diffusion there is not [Indiscernible] binding[Indiscernible] is what we need in this case. We also assume that there is [Indiscernible] tissue in the whole body distribution [Indiscernible].
In terms of metabolism, can use either clearance or more specifically if we assume that metabolism is not linear with use the [Indiscernible] For excretion we generally just use the [Indiscernible] . A lot of this information can be found in a paper by[Indiscernible] and others and it will describe the individual models and the parameters. Was interesting to note also is that [Indiscernible] in 2011 have described the different transfer doing tools that are available that you could use to parameterize these PBPK models and as an illustration [Indiscernible] in 2007 have used only *in silico* tools to parameterize their PBPK models for [Indiscernible] and did a pretty good job. The [Indiscernible] program also works with the first tier IVIVE models and use *in vitro* tools like [Indiscernible] to estimate [Indiscernible] compound in the liver.

I split up the *in vitro* tools that we could use for these PBPK models. You have a number of *in vitro* tools that have generally not been used that much for that purpose but you could use it for -- to estimate oral absorption [Indiscernible] has been used a lot in drug development. You could also use a simple [Indiscernible] system which does not contain [Indiscernible] but as a passive diffusion and you have dermal absorption you could use the skin permeation assay [Indiscernible] model which are also available. There are a few IVIVE studies available the estimate dermal absorption. You also have elimination -- inhalation tools in the headspace model is very common as well as [Indiscernible] which is not been used for these purposes but [Indiscernible] that you could use to assess the rate of uptake. For distribution, when you need to estimate partition coefficients I will go into more till later of what the type of *in vitro* test their art that you could use and that goes the same for protein binding in permeation coefficients.

[Indiscernible] metabolism is important in these assays and literature you will generally find [Indiscernible] used human [Indiscernible] to assess the rate of metabolism, rate of excretion but you could also use human of µ's and [Indiscernible] essays which will also be described later on. [Indiscernible] available also liver -- human liver [Indiscernible] excretion like I said is generally assumed [Indiscernible] there are some PBPK models that are available which could include your after uptake and secretion in the kidney for example for these to have [Indiscernible] properly verified. On to some of the examples of *in vitro* tools that are available that you could use to assess the distribution, up at three very common methods that we used in this field. The first one is equally preempting analysis -- dialysis -- equilibrium dialysis and you assess the [Indiscernible] between these two -- the concentration of the chemicals between these two in the equilibrium constant given an indication of printing hunting of tissue. There is also the author center for [Indiscernible] which has the advantage that you don't have a membrane in between so [Indiscernible] compounds do not stick to it. It is a very extensive -- expensive system [Indiscernible] sample with the protein and measure of concentration and then there is ultrafiltration which is a mix between the two.

What I have to appear solid phase micro extraction which is a new system when comes to estimating partition coefficients and we use this [Indiscernible]. It is a glass fiber surrounded by a polymer coating and only absorbed the unbound fraction of the chemical. So if it is bound to some perching it will not absorbed into the fiber and the nice thing here is that it is really a sampling extraction that to -- that it -- one[Indiscernible] which are harder to assess using the traditional method and can also use this method directly in *in vitro* assays to assess the concentration and these *in vitro* systems. And I will explain that later. Then there are a number of tools that you could use to parameterize metabolism. These include the different liver models

that are available and it is simple when it comes to [Indiscernible] and microsomes and whole body or tissue slices. Generally, what we do here is measure the appearance of a parent compound in these *in vitro* assays or we measure the rate of metabolite generation in these assays. Also depending on the need of your IVIVE model you would either determine specifically a KM and [Indiscernible] of the compound or [Indiscernible]. I'm not going to go through all the different types of [Indiscernible] that you came from these *in vitro* assays and we need to make sure that we understand how this relates to an adverse outcome. In this case for example in [Indiscernible] you might have an odd regulation of certain genes him need to understand the steps are and how this relates quantitatively to an adverse outcome otherwise we do not know the relevance of the *in vitro* assay in this case.

[Indiscernible] the one and discuss the challenges specifically for IVIVE and I put up a few of these challenges here to illustrate with the challenges are for the *in vitro* and *In silico* perspective. What is important limitation is that a lot of these *in vitro* assays have not been fellow for IVIVE specific purposes so the chemical applicability [Indiscernible] is limited tested or to find out and the biological applicability domain again in the form of an adverse outcome pathway is generally not well defined yet. The effective dose in these *in vitro* assays is not will determine. A normal concentration, the concentration number at to the system does not necessarily mean that is the concentration medicine is the target these assays and we need that concentration to related to a tissue concentration in IVIVE model.

There are a number of initiatives going on at the moment where we're trying to develop good modeling practices [Indiscernible] for these IVIVE models to verify the processes which was already discussed and we are developing [Indiscernible]. For practice guidelines in these cases to maximize the [Indiscernible] reliability credibility in the acceptance of these *in vitro* assays for parameterize think the PBPK models. Here we required minimum standards for sale in tissue culture and I put the two papers that relate to this. [Indiscernible] in 2011 discuss the guideline for subculture techniques and [Indiscernible] in 2008 where they discuss good modeling practices not only for PBPK models but can be extended for IVIVE models. On to what I would like to discuss now is *in vitro* dose metrics. This is a very important aspect within the IVIVE framework because as we -- just like *in vivo* the unbound fraction tends to be the fraction that is taken up in the tissues but we generally don't consider this in *in vitro* assays. And I will illustrate how that can significantly cause problems [Indiscernible] translation [Indiscernible] to *in vivo* because as you can see a compound that is added to the system at normal concentration the chemical can evaporate just like in the human body. It can bind to plastic, it can bind to protein, and then only a fraction of that will be taken up into the cells and a fraction [Indiscernible] something like a receptor. This can vary significantly between different types of assays and between different types of chemicals and this is what we need to consider.

Cell assay components determine the target concentration in *in vitro* assay set medium components can vary significantly we have different levels of [Indiscernible] serum for example and we can use 3-D matrix like an [Indiscernible] and these can also bind compounds. The chemicals [Indiscernible - muffled audio]. Will bind to the plastic and if the dimensions differ extent to which the [Indiscernible] binds the plastic will differ greatly also. [Indiscernible] cell density and greater number of cells [Indiscernible - muffled audio] assays also differ greatly in terms of the exposure times. [Indiscernible - muffled audio] and we expose them for days [Indiscernible] and then there is a temperature difference for a lot of these assays which will also affect the binding in the partitioning and these assays. You have a pH difference between the [Indiscernible] media and therefore also [Indiscernible] compounds will have different partitioning in a different assays. There is a great difference in metabolic capacity between the

assays in a great difference in transporter expression and we also even don't know how these relate to each other and how they relate to the IVIVE situation.

The fraction that enters into [Indiscernible] target will depend on the chemical. As well so different chemicals have a different binding affinity to things like serum, and have a different [Indiscernible] constant and will have a different rate of evaporation and different peak a a -- different solubility, different reactivity, different transporter affinity and different metabolic in some affinity which will all affect the concentration that reach the target in these assays.
To illustrate how much this can affect the *in vitro* readout that you have, I put up an example of [Indiscernible] here that we did a while back now and you can really see that if we use very little serum which is expected in the *in vitro* assays. The fraction the enters into the cells will be much greater and that can explain why you have so much more toxicity in these assays when you use less serum. We based our [Indiscernible] concentration on the number of concentrations that will be different in effect the medium affect concentrations that we measure sometimes [Indiscernible] magnitude. If we then use the free concentrations, the concentration of [Indiscernible] in these assays specific the dose response curves lineup and we can be more certain about the readout we have in terms of the toxicity. Then of course we have [Indiscernible] there is a difference in [Indiscernible] 13 for example and you can see there are a number of regression relationships available and literature that allow you to predict something like the partitioning in the binding to serum proteins in these *in vitro* assays. [Indiscernible] lower fraction will be available for [Indiscernible] and also the lower the toxicity you would expect.

These models are available and have been tested generally for [Indiscernible] chemicals and we are now looking at what this partitioning of these [Indiscernible] assays would be for [Indiscernible] compounds. Public dimensions -- like I said also play a large affect their riches are target and [Indiscernible] that you have the less serum in your system the Mark Levine to your plastic at least for [Indiscernible] and the more [Indiscernible] the greater the binding to plastic. [Indiscernible - muffled audio] open system so chemicals can evaporate out of it. A very volatile compounds this is a problem and we have tested specifically ourselves [Indiscernible] and as you can see if we use the nominal concentrations we generally use and systems [Indiscernible] we see that the effect concentration that we measure is 10 times greater than the effect concentration that we measure we maintain the concentration [Indiscernible] constant in these assays over time. So, here we [Indiscernible] type of volatile and [Indiscernible] compounds to use a different measure of free concentration with the constant flow of your compound. In this case [Indiscernible] that were exposed to compound and the effect concentration that we measure in these assays, correspond to much better to the effects, the lethal concentration that we find in these fish.

The exposure time is very important. [Indiscernible] and colleagues have looked at this extensively. The greater the exposure time in general the greater the toxicity that you observe for these assays until you reach way calls and [Indiscernible] it's important to see [Indiscernible] to observe and what the effect of this time is on the readout and then decide whether you could use that for your prioritizing the IVIVE model. The greater the number of cells the system compared to the volume of the medium the greater, the lower the amount of the chemical personnel so the lower the effect, the higher the effect concentration to the other shift on those response curves to the right and of course the greater the mix [Indiscernible] of the compound [Indiscernible - background noises] Affect that you expect.

As you can see we've done this with [Indiscernible] chloride which [Indiscernible] the greater the chain, of this compound the greater the toxicity. As we expect the concentration, the effects -- lethal concentration and sales [Indiscernible] cell lipid to see a huge variation of the toxicity

[Indiscernible - muffled audio] closer to the target concentration [Indiscernible - muffled audio] a lot of these *in vitro* assays are now using repeated dosing and what's interesting in these repeating dosing assays is that if we assess the concentration of the chemical in the medium because we just added every single day we don't see a great effect in the concentration [Indiscernible] between high doses and low doses. The format[Silence] Frost -- for all the assays that require for the IVIVE model [Indiscernible] there are a few modeling approaches in literature that you could use to quickly estimate what the [Indiscernible] just going to talk about one that we use -- this one is based on the data that I had sure you earlier. The [Indiscernible] binding affinity December 10 in the amount of stem 13 in the assay the plastic in the amount of plastics posted the asset in the number of sales -- sales and the amount of [Indiscernible] tran14 You can have several orders of [Indiscernible] differences in the concentration in the sale and across all the chemicals in this program you also see the normal concentration will be the same there will be several orders [Indiscernible] defenses in the concentration -- concentration in the sales. If we model the system [Indiscernible] we can do an okay job in estimating the [Indiscernible] in *in vitro* assays.

In conclusion, I would like to say that the data that is needed for IVIVE models is dependent on the required decision and chemical and exposure routes. We have to different types of parameters that need to be estimated that the physiological parameters such as -- and chemical specific parameters which we need to estimate using the *In silico* tools that are available. The partitioning parameters that we have such as tissue, blood [Indiscernible] can be estimated using *In silico* tools at least for the neutral organic compounds that what happened when you to expand them to other [Indiscernible] and we have cleared parameters which we don't tend to use *in vitro* tools for so this is really an estimate of the rate of [Indiscernible] cultures and then we also make sure that we defined the applicability the main of these *in vitro* and *In silico* tools properly [Indiscernible] extrapolation models and we have to consider *in vitro* kinetics said that the distribution of a compound as well is that from an *in vivo* system where we use the IVIVE model.

What that I would like to conclude. If you have any questions at this time please ask and I have to also say there will be a roundtable discussion for more specific questions.

[Applause]

Looking at the PBPK model [Indiscernible - low volume] When you were talking about the PBPK models you mentioned that some of the parameters like what is lower, pretty well known and others like partition coefficients, are more uncertain and I'm wondering why you can't express those different levels of uncertainty mathematically and come up with an overall constant level for your model.

Nynke Kramer
That is a very relevant and good question that is currently being discussed as far as I know. The problem with a lot of these *In silico* and *in vitro* tools is that they have never been to develop to be used for these IVIVE models so proper [Indiscernible] you could use the Monte Carlo simulation to express these uncertainties and give all these tools [Indiscernible] of evidence and there is [Indiscernible] doing this already and I'm pretty sure that is not the case [Indiscernible] well-defined in that case.

Richard A. Becker
Let me remind people on the web if you go to the chat area at you can type in your question and we will bring that forward for our speakers. Any other questions before we break?

Our program now calls for a 20-minute break from 10:10 until 10:30. Is that right?
So, let's take our break and then rejoin at 10:30. Thank you.

10:10 AM–10:30 AM Break

Richard A. Becker
It is my pleasure to introduce our next speaker Dr. Miyoung Yoon she is the director of bio connects and bio simulation at ScitoVation. She's a recognized leader in the initially quantitative leader of injury from December what -- [Indiscernible] she is the co-author of more than 35 publications and book chapters. She received her PhD in pharmacies from [Indiscernible] [Inaudible - static] on the subject of the quantitative IVIVE. She has done a number of educational training sessions in toxicology workshops recently. The folks on PBPK and [Inaudible - static] she is the member of the [Inaudible - static] she was speak on examples illustrating potential applications of IVIVE in chemical assessment. For those tuning in via the web if you would like to send a question in any time during the presentation or panel discussion I was reminded that you have to go to the chat box on the upper right-hand side. Make sure you select everyone so everyone will see your question. Otherwise, it may just get sent to the administrator.

10:30 AM–11:10 AM Examples Illustrating Potential Applications of IVIVE in Chemical Assessment--Miyoung Yoon, ScitoVation, Research Triangle Park, NC

Thank you for the nice introduction. I would also like to thank you for the opportunity to present on the potential applications of the IVIVE approach to support [Inaudible - static] We are having technical problems. I disclose that I have no conflicts of interest. The presented research today and case studies were possible because of the support from American Chemistry Council and the Council for advancement [Indiscernible] and human risk assessment. This is the overview slide. I'm going to go over some of the background at a high level because I realize it is a broad topic this field is evolving rapidly. I'm going to talk about IVIVE background paraben cumulated case study. Also use of PBPK model to address all the issues of exposure.

Since the to set the 2007 NAS report on Toxicity Testing in the 21st century there have been a disagreement to start in Europe and America to move away from the testing to pose the human relevancy in the toxicity testing and safety assessment. The overall goal is to establish safe human exposures based on cellular assays. I want to emphasize that the modern biologic tools used to predict mode of action based on *in vitro* assays meaning assessing the IVIVE in support with safety assessment relies on how good the *in vitro* [Inaudible - static] study is. Another emphasis is the use for competition law approaches assisting the translation of the chief surveys -- survey results in the context of human safety. This is something that Lisa described it. The traditional safety assessment.'s [Inaudible - static] the human safety information relies on toxicity studies which uses [Inaudible - static]. There are numerous in shoes about the stipulation of how relevant the predictions point of [Inaudible - static] for humans based on different species. As well as the [Inaudible - static] approach [Inaudible - static] human kinetics.

Under the new paradigm in the toxicity testing human safety exposure is now estimated largely based on the *in vitro* toxicity assays. In this paradigm how can we translate the concentration we put into this in a form that we can make risk based decisions? This is something that [Inaudible - static] should. I will make it brief. Before we begin I want to explain that what is quantitative in *IVIVE* [Inaudible - static] the quantitative *in vitro* stipulation is the whole process of translating *in vitro* assay results, immediate concentration in the context of safety. What is the

equivalent human exposure to the condition [Inaudible - static] the concern about the by activity testing compound.

The *in vitro* and *in vivo* stipulation is on a smaller scale. It is best -- based on the genetic assay results and [Inaudible - static] approaches. The human safety information or range of safe exposure and the nature of toxicity and potential target issue is coming from the toxicity assays. It is a snapshot of what is happening at the target size *in vivo*. These assays in heritability let *in vivo* processes in the human body [Inaudible - static] what matters for the dose response is that the concentration of a compound or entity of compound at the target site and then the actual dose based on raising the biological activity at the site of action. In this QIVIVE screen [Inaudible - static] to incorporate human kinetics into the consideration. Based on the assays results based -- assisted with the approaches [Inaudible - static] excretion parameters as well as the metabolite profile and describe human kinetics using the computation outputs using the [Inaudible - static] in combination with [Inaudible - static] we can [Inaudible - static] into a form that we can make a decision about human safety exposure.

Before we began to talk about potential opportunities using QIVIVE to support safety assessments these opportunities that are determined by or dependent on what kind of *in vitro* assay results or data we have. Or what is the purpose of using QIVIVE to support safety assessment . Obviously there are two different needs. Using higher throughput assays to prior to -- prioritizing chemicals for further testing. There are other needs to support product development and accurate individual compound risk assessment. Based on *in vitro* toxicity assays if you have a range of high throughput screening assays versus [Indiscernible - heavy accent] your focus will be determined by the *in vitro* toxicity results you have in hand. Screening or more accurate addition of -- prediction of safety exposure. Whether that data can enter about specific questions in the safety assessment. Using the former kinetic model and the nature of *in vitro* kinetic assays are determined or influence the potential opportunity we can have with the QIVIVE in the safety assessment. Remember we are aiming to use *in vitro* data to help determine the human safety of the chemicals. Which has a wide variety of properties. When using *in vitro* kinetic assays or dynamic assays you have to keep in mind that some of the assays have limited domains over [Inaudible - static] so depending on what kind of chemical space we are dealing with the QIVIVE opportunities differ.

Whether your purpose is helping to prioritize chemicals for testing or more accurate safety assessments, the common goal is to increase efficiency and human relevancy in the safety assessment by using more advanced biology tools of the *in vitro* [Inaudible - static] approaches. The IVIVE is a tool that can meet the needs for both sectors. Regulatory agency is [Indiscernible - heavy accent] and some agencies need to use *in vitro* assays to perform or conduct safety assessments for [Inaudible - static]. For the industry is more about developing biologically relevant assays for the compound they are dealing with so they can guide decisions and selection of chemicals for product development. They have to meet the regulation part requirements so both sides need to communicate. A decision example of domains applicability is an example with *in vitro* metabolism assays.

One of our research projects is focusing on developing new *in vitro* tools to better predict human metabolism. I wanted to use the metabolism as a as an example. On the far left is the *in vitro* assays -- assay choices we can have. Primary incompletion all the way to a more complex organic to pick culture in bioreactor systems. Fewer *in vitro* kinetic assays and the endpoint is [Inaudible - static] than as a result the chemical space you can cover or complexity of the PBPK model is limited for this kind of chemical [Indiscernible - heavy accent] because [Indiscernible - heavy accent] only last about a few hours. It is hard to address the [Inaudible - static]

compound. If it is a compound the [Inaudible - static] majority of determinants of chemical concentration changes *in vitro* then it is very hard to [Inaudible - static] what is the biological information. Then extrapolate to *in vivo*. Obviously primary incompletion would be limited to the [Indiscernible - heavy accent] appearance and appearance only. [Inaudible - static] [Indiscernible - heavy accent] complex model with extended endpoint then it will affect the applicability of the IVIVE models to support safety assessment.

You can see with the more simpler *in vitro* kinetic as a models give us the opportunity to use IVIVE for more screening and prioritization. You can have more constants using *in vitro* models to estimate the human safety exposure and conducting the safety assessment. Here is the representation of the application or prioritization using *in vitro* data in human exposure information with a margin of [Inaudible - static] Prioritization is dependent on where this compounds potential concern is. It explains human exposure to [Indiscernible] required for toxicity. That means exposure activated from the *In vitro* tox assays. With that exposure we consider it might be something to worry about [Inaudible - static] the green explains estimated human exposure from the volumes and other exposure prediction tools. If you see the large gap it means that your margin of safety or margin of exposure is larger compared to the right side of the example with a narrow margin between the concentration that may be causing [Indiscernible - heavy accent] and known human exposure. In terms of prioritization the right -- prioritization the right side -- prioritization [Inaudible - static]

This shows the implementation of IVIVE incorporating kinetics into symmetry in terms of using *in vitro* assay [Inaudible - static] assessment. Why do we call it simple IVIVE or [Inaudible - static] is that human exposure estimations based on *in vitro* kinetic assays is based on only two key factors. Giving us a good estimation of what human exposure might the equivalent to effective concentration from [Indiscernible]. But it is not more components of human safety exposure levels. The other side of the application is that the *in vitro* to *in vivo* extrapolation to support the *in vitro* based safety assessment that in this case *in vitro* tox assays is required that the *in vitro* assay should reflect the [Inaudible - static]

So the point of departure or estimation of reason of safety is relevant and converted to [Inaudible - static] to make decisions about human safety exposure. IVIVE or QIVIVE in this is not one of the other IVIVE can support both lower tiered testing as well as higher tier testing depending on the nature of *in vitro* kinetic assays. We can support the *in vitro*-based safety assessment with this *in vitro*-based prediction tool. In this testing approach the PBPK modeling plays a critical integration in making risk-based decisions. It is a bit hard to see from this illustration but incorporation of human exposure into consideration and the tools available to make that exposure [Inaudible - static] outcome is a tool to support *in vitro*-based risk assessment. The PBPK model plays a role as a integration tool that can coherently combine information from diverse *in vitro* and [Indiscernible - heavy accent] to address the aspect of the safety assessment.

Our first case study I'm going to present is quantitative *in vitro* -- *in vivo* circulation study margin of safety assessment. The parabens has concerns because of the wide used group of compounds as preservatives and a variety of consumer products including food as well as pharmaceuticals. The concern has been raised because of the potential of endocrine activity of on parabens. Based on the [Indiscernible] and based on [Indiscernible - heavy accent] The concern is the use pattern is based on the mixture so exposure to the make sure is a concern [Indiscernible - heavy accent] QIVIVE approaches. First, they develop the PBPK model based on *in vitro in silico* data. Estimates population exposure to this compound [Inaudible - static] using PBPK model developed using *in vitro* kinetic data combining with dosimetry and exposure

of the population. Margin of safety analysis was performed using *in vitro* dynamic assay results conducting the point of departure concentrate and in this case, we used assey 10 from activity screening assays. That concentration information was converted to human equivalent exposure using the PBPK model and then compared to the estimate *in vivo* blood concentration of parabens.

Importantly we wanted to identify the data gaps to improve [Indiscernible] because of the fact that *in vitro* assay tools also involve as a QIVIVE approaches. I wanted to describe what is required to improve or increase the constant using *in vitro* results to support safety assessments. The human PBPK model was developed using physiological parameters to describe the whole body is relatively well comprised in the literature. The 2007 study is something we included in the reference list. Those parameters we used to describe the physiological condition in adult humans. We used the *in silico* estimation of partition coefficients. [Inaudible - static] prioritization of the PBPK model. One area is more advanced than the rest.

The prioritization based on tissue components [Inaudible - static] advanced compared to prediction of metabolism. Which is problematic. It is based on the QSAR approach any need *in vitro* assays to estimate human metabolism parameters. That is why we estimate it metabolism parameters *in vitro* study [Inaudible - static] taken from the literature. [Inaudible - static] using metabolism beta because of the chemical properties metabolizing a reasonable timeframe to use those short-term assays. Most of the time *in vitro* kinetics and pre-consultation is not the issue. The dosimetry was performed using this *in vitro* parameter PBPK model to calculate the pre-plasma concentration equivalent to 95% of urine concentration based on NHANES. [Inaudible - static] [Indiscernible - heavy accent] point of departure was used with *in vitro* assay results. DC-10 based on human relevant cell lines containing potential screening assays. [Inaudible - static] as a ratio of *in vitro* or *in vivo* plasma concentration. Before we put together a PBPK chapter we wanted to describe the paraben metabolism in the PBPK model. The biomarkers are based on the paraben rate appearance compound. What we are interested in comparing in *in vitro* bioactivity data is [Inaudible - static] but the human biomarker for concentration is total paraben concentration including the [Inaudible - static].

Here is the PBPK model structure for paraben. The blueline indicates the biological [Inaudible - static] used. Which is the *in vivo* -- *in vitro* derived metabolic parameters. [Inaudible - static] one thing to note that there is a [Inaudible - static] happening and it is important depending on the exposure order. If you are describing the order exposure in appearance and testing it plays a huge role in determining the real internal exposure. The screen activities will include [Indiscernible] and a screen. We assumed simulated of toxicity [Inaudible - static] based on the observations and evidence cited in the 2015 paper that the cumulative toxicity was [Indiscernible] at the [Inaudible - static] level for the 17 compound mixture. We used a conservative approach for the parabens tested in this QIVIVE.

Point of departure something we use to on several paraben margin of safety with assay 10 paraben or [Inaudible - static]. Here's an example of marginal safety for human adult females. The assay 10 [Inaudible - static] then relative constants affect is that will we assume the future paraben is 100% potent than what is the relative activity of [Inaudible - static] so we express the potency as a fraction. The NHANES unit concentration was used. We estimated the human plasma concentration equivalent to producing the biomarker levels then [Inaudible - static] concentration for *in vitro* parabens as well as [Inaudible - static] we combined all the parabens. Then we compare DC-10 and the human plasma concentration. Because of the tool that we use PBPK modeling and [Inaudible - static] describing human kinetics is how we are able to compare apples to apples becomes [Inaudible - static] *in vitro* activity assays we are now

comparing in blood concentration as the target tissue concentration. We are comparing concentration to concentration and margin of safety is the gap between the assay 10 point of departure *in vitro* and general population plasma consultation to each [Indiscernible - heavy accent]. Both *in vitro* screening assays show a similar model of safety.
The PBPK model was successfully developed using *in vitro* metabolism data QSAR predicted partitioning. It was not in the sides but we used [Indiscernible] to evaluate the validity or appropriateness of using *in vitro* space higher taxation using IVIVE so we develop a PBPK model using *in vitro* metabolism data and we were able to reasonably describe *in vivo* kinetics using that approach. We use the same approach this time using human specific metabolism data and instructed the PBPK model. The rest is what we already discussed. I want to point out the constant limitations we identified during this IVIVE case study. Data gathered -- data gaps is really important to address the importance of improving the *in vitro* tools. [Inaudible - static] human metabolism because that is a [Inaudible - static] to constructing PBPK models to aid this translation. The other one is also related to human metabolism predictions. At this time it is by availability because of a pre-existing metabolism and parasite. To address this could data gap we are developing a new *in vitro* tool to improve *in vitro* tools as well as improving the QIVIVE approach and confidence in safety assessment. What we did is [Indiscernible - heavy accent] do not express the right [Inaudible - static] even though they are considered a standard for human [Inaudible - static] prediction. We estimate the model creating a new clone conducted *in vitro* obstruction and metabolism assays to estimate the pre-existing [Inaudible - static]. Then conducted *in vitro in vivo* extrapolation based on the [Inaudible - static]. We now have the submodel we plug into the IVIVE model. Briefly, the blue bar indicates the future paraben expected delivery based on the dose but because of the high level of [Inaudible - static] the reality it's mainly [Indiscernible]. Rather than the [Inaudible - static].

The other case study is addressing safety in the a PBPK approach. In order to describe the compound kinetics in different life stages three major elements that should be included is the dependent physiological changes and profiles of the metabolisms. Biochemical changes which is where we rely on *in vitro* based prioritization. Equally important we need to have a way to include age-appropriate exposure levels. The outcome of this approach is age specific. That will we can use to address sensitive populations and safety assessments. The challenges developing PBPK models especially for chemicals codes the [Inaudible - static] is based on [Inaudible - static]

If you want to use *in vitro* [Inaudible - static] to provide *in vitro* assays and also a challenge to [Inaudible - static] if we have to use individual donors. The approach here is [Inaudible - static] in conjunction with that information. The rate of metabolism coming from expressed in seconds and converted to *in vivo* metabolism considering the level of enzyme expression in the life stages. *In vitro* metabolism for IVIVE we use a parallelogram approach for [Indiscernible] and generally accepted approach use of [Inaudible - static] to describe disablement human express enzymes can [Inaudible - static] to identify active enzymes response rate and the data will be used to conduct the *in vitro* based prioritization and construct the PBPK model. Is used to evaluate the approach of them we have a case study for three or four pages to evaluate the same approach and identify the key parameters we absolutely have to conduct *in vitro* studies. With these approaches the final outcome would be the kinetic PBPK model supported with metabolism studies for each [Inaudible - static] with the human relevant exposure prediction tool and use this to [Indiscernible].

IVIVE workflow using the expressed enzymes starts with *in vitro* metabolism measurements that measure metabolic rates for compounds [Inaudible - static] and I want to mention that this particular case study we had careful about considering the bio candidate preconcentration

because of the [Indiscernible] was hard to determine for this compound. In combination with [Indiscernible] using the PBPK model and we estimate metabolism framework in exposure across [Inaudible - static]. I want to point out that equally important as defining preconcentration it is also important to define the *in vivo* preconcentration. [Inaudible - static] before we carefully reviewing consider what we are extrapolating. In this high-level compound it is possible that the [Inaudible - static] will be included in the protein assembly process and parasite because of their chemical properties.

Instead of getting in directly it goes through the [Indiscernible]. The compound protected within these proteins which is [Indiscernible] for the [Inaudible - static] that we anticipate *in vitro* for *in vitro* observation. Putting it all together, that approach can be used to predict target tissue or blood tissue concentrations. It can be used for [Indiscernible] and can be used to detect chemical specific adjustment that can replace [Inaudible - static] or can be used as margin of safety analysis. The goal is to improve the safe exposure guidelines for all like humans. We may need to use different thinking about PBPK models for safety assessment under the new paradigm. Now prioritization is based on [Inaudible - static] and evaluation is not necessarily against *in vivo* for any more human data. It's a validation of our approach and should be more based on the *in vitro* models relevance and quality of *in vitro* models. As well as the validity of [Indiscernible - heavy accent] used to convert that information to support the *in vivo* model development. Variation using the human data is valuable but it is more about opportunity then requirement. That I would like to acknowledge my colleagues. Sorry I went a little over.

[APPLAUSE]

Richard A. Becker
All -- I think we started late. It is not all on your shoulders. Rather than have questions now please hold your questions for the panel discussion. It is my pleasure to introduce our next speaker, Dr. Weihsueh Chiu who is a professor in veterinary [Inaudible - static] at Texas A&M University. Prior to joining Texas A&M me worked at the US EPA for 14 years as the chief of the [Indiscernible] [Inaudible - static] national Center for environmental assessment. [Inaudible - static]

11:10 AM–11:50 AM Opportunities and Challenges for Using IVIVE to Improve Decision Making--Weihsueh Chiu, Texas A&M University, College Station, TX

It is my pleasure to be here. I appreciate the opportunity to talk to you about the challenges and opportunities for using IVIVE in decision-making. The speakers today touched on both the basics of PBPK modeling how we generate data specifically for IVIVE and some case examples of how IVIVE has been used in the context of safety and risk assessment. [Inaudible - static] to see how we can advance the use and application of these tools. Aspects I am going to talk about is these six various opportunities and challenges related to using IVIVE and reverse kinetic modeling.

I'm going to frame my discussion on this basic outline of how IVIVE and reverse toxic kinetic is conducted. There is a part where you generate the data to put into a IVIVE model. You have a specific model and the parameters you run you eventually predict some sort of concentration or internal dose concentration that you then compare with what you derive from *in vitro* assays. Dr. Cramer talked about the data generation and how we use various techniques for collecting this type of data. Dr. Sweeney talked about the six models and how they are constructed and evaluated. Dr. Yoon talked about actually putting this together in a couple of applications. The first challenge [Inaudible - static] I'm going to start on data generation. Data generation for

IVIVE models is certainly higher than what is used for traditional PBPK models where you need to have *in vivo* data in humans and experimental animals. And experimental animals you are measuring time courses so you may need multiple species, different life stages, etc. There are data available for over 400 chemicals, which is more than traditional PBPK models. Not as much for drug but they are mostly using a classical compound -- model. Generally, the data is easier than building 10,000 PBPK models for extrapolating *in vitro* assays. It is not as [Indiscernible] as these *in vitro* assays. We have *in vitro* data from over 10,000 chemicals. We're not going to be able to [Inaudible - static] development of this data for IVIVE whether it is metabolite data [Inaudible - static] part of this is as opposed to in the screening context where you can find out how much your putting in based on good practices to do these types of assays you need a analytic method for each compound. Each compound often has its own analytic method. It is very difficult to parallelize this for a high throughput data generation system.

How do we bridge this gap? The number of compounds we would like to have IVIVE data on and the ones we can realistically generate IVIVE data on. What we need to do, IVIVE one of the main uses is to prioritize chemicals in terms of safety or risk their now we need to further prioritize the data generation for assessing that safety or risk. Prioritizing the prioritization. One approach is we have data on over 400 chemicals. We can use that as a what-if. Without the our 2K data would is the possible [Inaudible - static] for the relationship between [Inaudible - static]. And taking a worst-case scenario for what the chemical properties or metabolism properties are, if we have a large margin of exposure in safety [Inaudible - static] then maybe those our lower priority. The important part about prioritization is we need to know the decision context. The context in which they will use the data out from before we can apply this approach. We need to know what kind of *in vitro* assays we will apply IVIVE to. And then exposure estimates to get a rough margin of exposure estimate.

Going beyond taking the worst-case, you could have more sophisticated prioritization schemes. You can try to divide chemicals into various classes. An approach where you have based on clinical properties and structure you can classify them into different concentration categories. You can also apply QSPR to get statistically based [Indiscernible] [Inaudible - static] this is approach developed by colleagues of his day EPA. The basic approaches to first look at what compounds you can have realistically generated *in vitro* toxic kinetic data from. Then based on chemical properties look at which ones would realistically reach steady-state within the timeframe you are interested in terms of the toxicity.

These ever hear the assay has failed or things you expect based on the chemical property. The traditional using GSR in free concentrations or using the usual metabolism assumptions are not going to apply. Then divide them up into groups of chemicals for which you can easily apply IVIVE. Then once you cannot do any type of IVIVE without some type of *in vivo* data. Again, we are prioritizing the prioritization. You can afford to have a little more uncertainty because you are in the prioritization context. Second challenge addressing variability and system ability has been touched on the a little bit. The typical data uses old samples. [Inaudible - static] there is no real information. Often it is difficult to get information about a particular life stage. Other types of our 2K modeling have a lot of good information on physiological variability's. That information has been collected over the years to form original PBPK modeling and for use in the IVIVE context. These often make assumptions about variability related to clearing or other specific parameters. It assumes a 30% [Inaudible - static] compared to some of the other models like the package develop [Inaudible - static] 30% clearance variability and [Inaudible - static]

Is that adequate for this type of function to capture the amount of variability and susceptibility we might expect in the population quick one way to look at this question is to look at the historical

data on [Inaudible - static] in humans. This is data much of which is owned pharmaceutical. 430 chemicals for which there is data *in vivo* for which individual data were available in the published literature. There is a distribution of the ratio of the 95th percentile in the median as a toxic kinetic variability factor. The typical values are around to but it goes up to maybe five with a little variability. These are limited to mostly pharmaceuticals [Inaudible - static] and mostly healthy volunteers are between 20 and 50. There are limitations in the data but it still provides useful contacts. Do they provide a reasonable range of variability compared to what we have seen *in vivo* and in humans?

Looking at our 2K data for 400 chemicals you can get a simpler distribution for the 96 percentile in median individual. The center of this distribution across chemicals is about a two-fold variability factor. The upper [Indiscernible] is a little more than two which [Inaudible - static] *in vivo* data there seems to be some chemicals with much more variability. Maybe some of these assumptions held into the models are potentially missing some chemicals with higher PBPK variability. What can we do? One solution is a bottom-up approach to look at variability across subgroups. The example with [Inaudible - static] was a good illustration.

This particular study is based on work done by Whitmore in others when she was at [Inaudible - static]. The idea is the original our 2K approach is to take [Inaudible - static] and run it through something and you will get the plasma concentrations for the general population. Now what is being used is metabolic enzymes which is the assay for each enzyme which is a puzzling you put together enzymes into a virtual lever for a particular lifestage because there is information in the literature about the activities or concentrations of different enzymes in the elderly and various different ethnic groups. Then building up your total hepatic clearance based on summing up the clearance from the individual enzyme. [Inaudible - static]. In this work by Whitmore and colleagues they went to nine different chemicals and looked across various different subpopulations looking at the most sensitive population and most of them were neonatal. These are ones [Inaudible - static] they do not come online until after the neonatal period. A couple are primarily [Indiscernible] populations were ones that were most sensitive. I know this is difficult to read but the estimated variability here is a factor of seven or factor 11 and these are much larger than the our 2K estimates showed earlier. Those are based on generic assumptions as a result metabolic variability. The contribution of these differences in terms of possible reasons like the fact that the change with age and they change with different subpopulations and contributes to a large percentage of the overall variability. In many cases upwards of 80% of the variability is due to these differences across subpopulations. These are differences mostly based on the chemical specific factors and not based on the physiological factors. These models also take into account [Inaudible - static] at older ages as well as deficiency being related to the declining of age.

This previous approach was prioritized as chemicals [Indiscernible]. This is much more work. You have to do assay experiments across many different enzymes and for each different chemical, you need to do the chemical analysis. We can basically take the methods that have been used in more pictures additional modeling and apply them in the IVIVE context. *In vitro* data with [Indiscernible] enzymes was used. Literature data was used on the variability of content and liver weight across the general population. There is variability in enzyme content in the healthy population. Energy -- in addition there were [Inaudible - static] variability using whole liver extracts. As an example, this is to minimum men, women, and putting this into a PBPK model is a twofold range from the 95th percentile to the median in a threefold range from the 99 percentile to the median. For a chemical, you can use what we've learned over decades about traditional PBPK modeling and apply it to look at chemical specific variability.

The third challenge is talk about uncertainty. We collect all of this data inputted through model. We have the parameters so what is the ultimate uncertainty in our results. This is now different from the traditional PBPK modeling. For traditional PBPK modeling MS cases we have *in vivo* data in animals to validate or calibrate the model against. Here we are working in a clearly predictive mode in which we do not know if they have any data in humans on the intake concentration relationship and all we can do is compare with the subset of chemicals and look at traditional measures -- PBPK models or compartmental models. This is from [Indiscernible] 2015 paper. Looking at predicted concentration versus the actual concentration reported in the literature. The pharmaceuticals are in red. [Inaudible - static] most of those are closer than some of the other chemicals for which there is a few. You'll also notice these are [Indiscernible]. There is an order of magnitude that is not unusual amount of uncertainty or predicted error in the estimates.

It could be more formally incorporated into IVIVE estimates. It has not been done. Alternatively you can adjust the margin of safety in exposure that takes you to the next testing based on the fact that you know they will be uncertain to in order of magnitude or more. The fourth challenge is the domain of active ability. I will not spend time on this because the previous speakers have discussed this. There are many examples of developing more find PBPK models or more refined data. Toxic kinetic data based on the different times of assays. Dr. Kramer had mentioned beyond hepatocytes. The limitation of the typical simple our 2K approach is that is a simple compartment model. Always talking about the compound not metabolites. You are assuming it is in exposure scenario where it jives toxicity. We're only doing one compound at a time. We can probably talk about it more in the roundtable discussion about the domain of active ability issues. The fifth challenge I would like to discuss more detail is the new models in the fact that we need to adjust how we do our IVIVE and tailor it to more specific type of *in vitro* [Inaudible - static] that we want to do the stipulation for.

One of the big areas that will require a lot of thought is our future with tissue chips. It is a generic name for different types of some this dictated *in vitro* systems from 2-D cell cultures [Inaudible - static] going into more sophisticated micro-physiological systems where there is actually a flow of media through the system. Often in 3-D. This is very important for a lot of tissue types. The share stresses and how the cell responds. Things like the lungs or kidneys. Philia and [Indiscernible] extends the flow. Back can affect the expression of the transporters and activity of the transporters in the cells. Having this micro-physiological system in which you have an actual flow through the system to mimic that share stress can lead to more realistic assessments of potential toxicity. This goes on to more sophisticated approaches where there is multiple cell types. On a single platform to the idea of a human on the ship where we have different organ ships we connect together using micro-physiological flows so you potentially have a [Inaudible - static].

If you want more information you can go to the National Academy of Sciences workshop on the potential for [Inaudible - static] in environmental health. Also part of in age there is a program devoted to tissue chip development in collaboration with discernible -- [Inaudible - static] traditional IVIVE has been based on serum concentrations and equating the media concentration with the *in vivo* concentration. These tissue chips may require a different type of estimate whether it is a portal of entry to symmetry which may be more of a surface area issue rather than concentration concentrations may be more relevant than [Inaudible - static] the message is regular concentration there is concentration in the particular platform that may not correspond to usual IVIVE models. Another issue is that the concentrations may not be directly accessible for [Indiscernible]. You can always measure the concentration in the well but these tissue chips are often closed systems. You cannot actually go and see the actual concentration

in the chip. You can get concentration going in and concentrations going out. Maybe there were other sampling ports in there but it may not be directly accessible. Meaning you may have part of your IVIVE model [Inaudible - static]. Similar to the idea [Indiscernible] in terms of modeling within a different partitioning within a traditional *in vitro* well this would be more complicated because with micro-physiological flows you will need to be able to model disclose in addition to the actual human blood flows.

A second area for the potential to use is to extrapolate population-based *in vitro* models and this is based on work in combination with [Inaudible - static] as well as a student. The idea is they took [Indiscernible] from the thousand different outlines put into a *in vitro* system in which [Indiscernible] was measured as the endpoint. Then look at the chemical population and tested with 170 compounds. As you can see for some chemicals the dose that which [Inaudible - static] begins to manifest is in a fairly narrow computation range. [Inaudible - static] their abilities over chemical dependent. The idea the original experiment was to identify a toxic dynamic variability factor. Which is the difference between the median and 1% individuals. How do we then apply IVIVE to this type of experiment? It goes back to the traditional RFT paradigm in which we provide a human variability into the dynamic factor. Whereas this kinetic factor applies to the *in vitro* population variability data applies to the toxic dynamic factor where as is toxic kinetic whether it is our 2K's model or PBPK model that applies to this toxic kinetic factor. How do we combine these? You combine them with uncertainty data. That is very simple and considered one issue you're not considering what population you will end up. The person [Indiscernible] proposes to claim considering these are independent. If you can assume this you can do this in a spreadsheet whereas you would need to do a prioritization in order to combine assuming [Inaudible - static] and you can add assumptions and correlations as well.

In this case our combined it [Inaudible - static] if you are aiming at 99 percentile it will not be 30 because it is the same person in the 99 percentile here and it will not be the same as here. Another issue is mixtures. The big advantage of *in vitro* screening is you can test metrics much more easily than you could in *in vivo*. This is an example using the population toxicity measure as a measure of toxic dynamic variability. Looking at a mixture of spastic assays. One is a mixture based on collecting environmental samples. This is a environmental mixture of pesticides. The second is a laboratory mixture of 36 different pesticides currently being used. Each mixture was texted *in vitro* and diluted to get a concentration in response estimate. How do you do IVIVE for a mixture in terms of *in vitro* study?

Again the usual approach. What they did in this particular study was did it one at a time. They did an IV for each component to make sure. Using the same types of data and models. There were some for which there was not our 2K data. The current under several met were five chemicals mixing and actual pesticides. Six out of the 10 chemicals were mixing. So the prioritization approach [Indiscernible] and they took two scenarios. One was a worse case where they assume there was no clearance and height protein binding. Therefore it was a slowly cleared compound. Then they weighed the mixture by percent in terms of the relative mass of each component within the mixture or equal weighting of each component of the mixture. Because you are not sure what is driving the toxicity. You have no idea which component is driving the toxicity. This is assuming they are similarly potent and waiting by equal weights assuming [Indiscernible] to their contribution. The worst-case scenario is one scenario and the median where you [Indiscernible] central estimate of the compounds that you have our 2K information on. Use that to do the *in vivo* for the chemicals you're missing. Then the [Indiscernible] dose in terms of total milligrams. Kilogram was -- kilogram per day was calculated based on these assumptions.

This panel is the different types of weighting schemes and different assumptions as to -- the two different weighting schemes based on the different assumptions and mixtures. One is the current use in one is the environmental make sure. This is the range across the different outlines. This could then be compared with the actual and predicted exposure levels. Here is the actual current use mixture. This is taking the pesticides and doing a laboratory to make sure there is a margin of safety -- the way they did these terraces was assuming the difference between the talks of [Indiscernible] variability and combined.

This mixture illustrates a number of different challenges. For the data degeneration there is missing for some components. Take a medium assumption and worst-case assumption compared to the data you did have. The domain of echoed ability and how we can do this for a whole mixture and the approach was each component in terms of the PBPK and -- assuming no interaction between [Inaudible - static]. Having a *in vitro* population-based model allows [Inaudible - static] and these were combined assuming independence. The final challenge to talk about is how we do this more routinely. How do we make something transparent enough so we can be used in a regulatory application? Ideally, this would be an open-source. This is one disadvantage. There are a lot of things in the black box that [Inaudible - static] regulatory protected might not be [Inaudible - static] and it is tailored to different audiences both for regulatory sciences as well as research scientists on the [Indiscernible] call. I see them having developed workflow tools based on a couple of online frameworks. In which these would all be put into a single toolbox together. Boasts the day that and the *in vitro* points of departure. Clearance per diameters or other metabolic diameters. Models will live inside this workflow. Tools for evaluating the models as well as actual output and results of the IVIVE extrapolation.

You can talk to Nicole for more information but they are anticipating spring of next year to be publicly available. Just to summarize the key challenges all the aspects of our TK there are a number of challenges involved in each of them. I think this will be a wonderful starting point for our roundtable discussion. In terms of challenges [Inaudible - static] some approaches of variability insensibility. Certainty is something that is only qualitative. The main out but ability there are a lot of experience with [Inaudible - static]. I think the tissue tips will present a major challenge going forward but for other types of population-based models the tools are there. For the workflow and transparency there are tools being developed and hopefully they can be test driven in the near future. Thank you very much.

[APPLAUSE]

11:50 AM–12:50 PM Roundtable Discussion--Richard A. Becker, Moderator, All speakers

Richard A. Becker
Going to ask our panelists to come up to the table. As we are getting settled let's remind people on the webcast if you like ask question now is the time. Submit your question to the chat box. Lisa is doing double duty as Co-chair and speaker. She will be monitoring the chat room for particular questions. Thank you for volunteering for that Lisa. I'm going to open it to the audience for questions. Any questions at this point?

Audience Question
Dr. Yoon, congratulations. [Inaudible - static] in my opinion. A.k.a. toxic potency in chemicals sometimes [Inaudible - static] and this toxic potency has a range of nine orders of magnitude. How we get to safety in the regulatory agency. We do that by using animal studies. To get the potency of the chemical safety. We try to extrapolate the humans. My question is everything [Inaudible - static] in your model rests upon your estimation of point of departure using *in vitro*

data. In this case that is EC 10 for paraben. -- EC 10 That is essentially the entire ballgame. How well you do that in the uncertainty is everything, I do not understand all of these methods [Inaudible - static] what is the uncertainty in it?

Miyoung Yoon
Interesting and good question. A paraben case study is not *in vitro* point of departure because as I mentioned as -- in order to define adversity or a safe reason based on *in vitro* we have to have advancement and development in the *in vitro* side. We need to have better *in vitro* tools to make decisions to be sure we are addressing the key biological processes in these regions that we will not see any adverse defect at the cellular level. We're not talking about actual point of departure for endpoint, I think it it is a new way of approaching [Inaudible - static] it is not really all about this point of departure do you see any effect [Inaudible - static] so absolutely to maintain [Inaudible - static] that is why wanted to move away from the risk and try to use the safety. You are merely extrapolating the core dose responses with the *in vitro* team. We want to use those estimated safe regions and then use that information to define the range we can safely say humans [Inaudible - static] I hope this answers your question. The example is if we assume that is a point of departure then we can follow this as a workflow.

Audience Question
My congratulations were correct but you have not got to the goal yet.

Richard A. Becker
Let me clarify, the point was you cannot define adversity and be true but alive Daschle biological effect you can use as a point of departure and that is a bit of a difference and you're not trying to make the leap that the point of departure *in vitro* and *in vivo* adverse affect it corresponds to biological activity *in vitro* and and the concentration and exposure and people and in humans.

Miyoung Yoon
That is correct.

Audience Question
The assumption is if you're well below the concentration than unlikely adverse effect and from a prioritization and screening point, that is probably adequate and if you overlap then you have a tiered approach going to the next level and start thinking about what potential adverse effects might be occurring and what are other essays that whether it is *in vitro* or *in vivo* that you need to have it to your testing approach.

Miyoung Yoon
Also want to add that using [Inaudible] to integrate those affirmations and up to a certain level that says maintaining no more functions and that should be the basis of defining safe regions.

Audience Question
I want to build on Ron's comment and Dan's earlier, I think again it is exciting to see something like a margin of exposure come out of the *in vitro* data but the critical that is necessary for supporting revelatory decisions about safety not just prioritization or screening. Will be an integration of uncertainty that is involved in different *in vitro* models and parameters being put into that. Somehow integrated and quantified to be attached to the margin of exposure because what we do now with animal data is to attach a number of safety factors to protect against their ability with inner species and among individuals and need to have comparable. In order to have confidence that margin of exposure is adequately protected and something you eluded to that right now it is qualitative people in the sense of what some variability might be but we need to

see that quantified in a well-accepted widely shared way in order for it to support decision-making. An example of a compound ANB and an enormous margin of exposure which is next to the smallest but it seems if you're trying to make decisions on these *in vitro* or in silica-based exposure, the most promising place where they can be the weight is where margins are the most enormous. In addition, not try to understand how much additional protective safety factor you need to add. That is something that will be of great interest and the ability to take that margin of exposure and somehow quantify that or understand the amount of uncertainty so you can attach appropriate vectors to make a safety decision whether or not to permit something or not at a certain level. This is exciting stuff.

Richard A. Becker
Thank you. Maybe composed that part is a question I think everyone alluded to something explicitly that it appears there are different decision context for use of this information. And her approach and perhaps it's time to or appropriate for folks to think about the decision context. You said it first, upfront and it helps you understand the greater more or less what degree of confidence you have to have in perhaps the panel can comment about in this gets to be applicability or sooner rather than later that the use of these tools and thoughts on the decision context upfront and how things are shaping up for different uses in different decision context.

Lisa M. Sweeny
The tiered approach to how data will be used is that the beginning and the affect data and any affect is a concern at baseline and you know you're being protected because they serve individual isolated processes and what responses may be that mitigate or limit how much impact it has on ethical in point where you see at a lower tier, you're trying to avoid missing a compound that could have an effect. Move along and when you're trying to go to a higher tiered more refined use of data that you want to know, how uncertain is it and that you want to be more precise and the triage approach of, how good are we or how bad can it be and by building expense with additional chemicals you know which ones are productivity, where it's good and look at the ones where it's bad and say what tools do we need an what do these have more -- have in common or enhance our *in vitro* approach to capture what we use on these chemicals and my experience from having intended a [Inaudible] conference several years ago that they would focus on whether tools didn't work so they can make them better and be more productive in the future and you see that with IVIVE application in the coming years .

Weihsueh Chiu
I think it's also useful to think about it in two different ways, with going from internal [Inaudible] to external and there's another IV going the other direction and from and *in vitro* effect to [Inaudible] affect and [Inaudible] raised in terms of what do the EC 10s mean and there's an IV in both directions and [Inaudible] context going backwards to what is the relevant dose. Into other applications, it will be increasingly important as well.

Lisa M. Sweeny
The next buzzword is quantitative adverse outcome pathways because they think adverse outcome pathways are helpful in delineating the steps but in order for quantitative use and risk assessment, that is where we need to go to take *in vitro* components and individual steps to figure out what does it mean to an integrated system as in with pharmacokinetic, the metabolism and lever wants to get to a certain point, it doesn't matter how much faster the rate is that the blood supply is limited and you sure the bottleneck and in terms of the adverse outcome pathways we don't have a very good sense of where the bottleneck is in the key events.

Miyoung Yoon
I think in terms of EOP, it goes back to the point that because of huge success in terms of using [Inaudible] screening [Inaudible] and now we know the data gap and use for improvement and improving [Inaudible] is one of the key areas that we need to improve in order to increase the confidence in using this approach for [Inaudible] safety decisions and again I think we need to think in a different way from traditional way of understanding [Inaudible] *in vitro* testing is not about replacing the toxicity but understanding the human biology and how it works and so when we see the dose response, with the right *in vivo* biology, then we can say instead of not necessarily going down to [Inaudible] at the final effect, we can confidently say, we can use the concentration [Inaudible] and hello this concentration we can make a conservative decision about the human safe exposure.

Audience Question
Thank you to the speakers for their presentations. You talked about mixtures and in the environment and you mentioned there is an assumption no metabolic interaction between the [Inaudible] mixture correct and you also noted it is *in vitro* methods are helpful for prioritizing or screening picture of concern, some of the mixture and competence may have [Inaudible] affect. However the default approach that is used is [Inaudible] . How would that IVIVE approach differ if the competence of a mixture are interacting because we might be overestimating or underestimating depending on how the competence of the mixture interact or has that been [Inaudible] . But I was curious in terms of the area [Inaudible] in screening and IVIVE and if there is a consideration in that direction.

Weihsueh Chiu
In terms of IVIVE, the approach that I talked about was taking each component and mixture separately. And at low exposure levels, you expect essentially the clearance and on those things are linear and the interaction terms would be that very large if negligible. And I think the more difficult interaction and from *in vitro* systems you begin to test [Inaudible] verse [Inaudible] paper because you can do things in [Inaudible] and test both individual compounds as well as various mixtures of them more effectively and maybe tease out statistically [Inaudible] for something more complex might be appropriate. But I think on that IVIVE kinetic side, [Inaudible] one at a time, it is maybe panel members had other suggestions to something that might be more sophisticated?

Richard A. Becker
Others in the panel that would like to comment?
Dr. Kramer?

Nynke Kramer
One thing we try in the lab is that you can expose things in a mixture and forget everything and we assume [Inaudible] bit it is a quick check to see if there is [Inaudible] may affect your looking at *in vitro* and a quick check to see if there's a mixture affect.

Weihsueh Chiu
The only other thing I can think of is some more untargeted approaches like Expo zone approaches and end of day might be adaptive or do an AV ABE approach. Because when you talk about hundreds or thousands compounds or something like that, one at a time it will not work either.

Richard A. Becker
Before we go to a question in the audience we have one from the web. Let me see if I can summarize and ask a panel for several to consider this and basically the question is of course we all know, given cultures represent a felt language is not the same as an actual cell in *in vivo* and organism. The question is whether studies that compared the dose effects *in vivo* to those used in the two systems into help document the relevancy of the *in vitro* systems. And the parallelogram type of thing and if so are there good examples that you can point to with cell types for particular test systems.

Lisa M. Sweeny
I don't remember if it was in a paper or presentation that I saw recently but I do remember some of the comparing what would be the key FX with *in vitro* results versus *in vivo* and seeing how good the correspondence was for different target organ systems. If you open [Inaudible] and my recollection is in some cases that there were some instances where work and some did not and that suggested there were certain extent the *in vitro* system are less adequate for certain endpoints and one that I think you remember was narrow, the current [Inaudible] endpoints did not seem to be the *in vitro*, not currently predictive of *in vivo* but I regret a don't remember the specific reference but I don't think there is a large literature in this area. An area for future exploration.

Miyoung Yoon
Because of the known concerns about the use of less reflecting human biology, there is that Dr. Chu mentioned, [Inaudible] link tissue [Inaudible] that other *in vitro* assays can compensate what is missing and [Inaudible] that is supposed to be reflecting the target site affect.

Audience Question
[Indiscernible - accent speaker] have given any comments using [Inaudible] and how different are those in terms of metabolism [Inaudible] . Do you have any insight into that?

Nynke Kramer
There are some of the ledger but not from an IV point of view. Depends on [Inaudible] that there are alerts and compound doesn't work for this system but I will let her answer.

Miyoung Yoon
In vitro metabolism is the parasite [Inaudible] for limited amount of time frame and can be a good reflection of human biology in the [Inaudible] but after viewers it lose the metabolic [Inaudible] and people are trying to develop more advanced than complex [Inaudible] and 3-D type of modeling to maintain phenotype of *in vivo* cell functions. Using flow-based models or stimulus from other sites, [Inaudible] it can act more like *in vivo* system.

Audience Question
As I discussed with folks during the break one of the reasons I came here today was that been working in this field for about 30 years and Dr. Sweeney mentioned there's nothing new with taking *in vitro* data and trying to construct data is to stimulate what was on *in vivo*. And my whole time of work, I am excited to see that we talk about that in this agency and what I suggest is we are not going to replace *in vivo* testing entirely with *in vitro* testing anytime in the foreseeable future but making progress meant for people to recognize *in vitro* study results inform *in vivo* study results and anytime we can get *in vitro* data supporting any *in vivo* results in regulatory purposes it's helpful to know how to do it and I think this education that we are getting out of the summoner and people studying in this field, it will help that process. Reduction, refinement and reduction does refinement -- reduction in refinement in animals. They don't want

us to use animals. This is a necessary part of the process learning to incorporate *in vitro* data into [Inaudible].

Richard A. Becker
Before we go to another question, I had one that is percolating and a top level. As we move towards more model base approaches and I think I heard a couple people talk about this and I have experience with it. Challenging sometimes to recapitulate a model from what you find in the published literature. Is there a need and activities underway to have model repositories and areas where you can better or where researchers can better exchange the type of information or find that information and build from what someone else has done because it is open access?. Other activities like that and I think Dr. Chu mentioned there something going on.

Weihsueh Chiu
They're looking to develop a conference of IV workflow and it can be reproducible rectal bowl and honorable essentially and in the future. And there has been other various efforts it popped up but it's hard to maintain steam on the and [Inaudible] who has mentioned earlier, you develop the model generator which is able to translate models between [Inaudible] and was trying to set up a model repositories but without a lot of buying or journals, you have a repository like they do for genomic data. It's difficult to heard the cats together to do that.

Miyoung Yoon
Over the course of collaboration with EPA and in TCP, they are collecting the [Inaudible] models and human data and started such an effort to making victory of PDP K miles that is available so far in using those to reproduce or [Inaudible] model approaches .

Lisa M. Sweeny
I know I have received emails from various groups collecting models but it tends to [Inaudible] and flow in the long-term viability especially with changing availability of different types of software, particularly challenging. I usually, if I'm interested in using a model that someone is developed, I go to them and go to the sponsors of the study and that is generally the first approach as to looking to go to a repository.

Miyoung Yoon
We had our workshop at JRC about what is the key challenge in accepting *in vitro* based PDP K models and one of the areas we have to move or make an effort is collecting those PDP K miles is that we have a good place to start and use that as a research to make export community to spread the word out.

Nynke Kramer
There was actually a meeting two weeks ago were returned to find the problems we run into, model verification and code verification are a lot of other areas and engineering is quite set on the idea that we do the same for every IVIVE. And come up with a guideline on how to do each step [Inaudible] and that would also ideally be whenever we publish something that it was in front of the guidelines.

Richard A. Becker
It was for two it is that I opened my email and it was from OCD indicating to see is putting forward a proposal in a deed for guidance document think characterizing and validating and validating [Inaudible] models for regulatory use in focusing on the types of models we talked about today where the generation of information and not the traditional models but *in vitro* models or other approaches and that's obviously a hot topic right now and a driving need to

have collaboration globally on how to gather the information and use it and help people who evaluate it and better understand the strength and limitations. That is an active area going forward shortly.

Another question from the web. The question dealt with human tissue on a chip or ex vivo or human organs or tissue slices and what role they can perhaps play in terms of helping in the IVIVE and improving for safety evaluations and I think the question specifically on the tissue and chip would be how do you get a chip that can be predictive for a quantitative assessment?

Let's think about that. I have another question, I think it is one that is an interesting one from the standpoint, with kinetics and dynamics and I think Dr. Kramer mentioned in her studies, she found there is not the same but simply measuring or using the nominal concentration and assuming that is the cell or systems are actually visualizing or seeing that in fact you can have finalization and finding to the matrix. Is that something that is a big hurdle to overcome or something that we can manage in a more systematic way? I'm thinking if it is a big hurdle that you can actually do analysis for every chemical in every system to understand the concentration that the inter-cellular concentration is a big challenge and other ways to overcome that?

Nynke Kramer
I think also in this case we do appear approach or approach where we apply alerts and if there are few models available in the partition model and actually new ones being made that would actually be applicable to things like a [Inaudible] system where we collect many PDAs. We follow it over time. Is a TK model and ideally, the chemical has [Inaudible] or chemical domain and we should consider that the nominal constraints [Inaudible] and decide whether this is enough to do the experiment or that we actually will do internal measurements. Again it is a tiered approach where you have an alert to correct system in place.

Lisa M. Sweeny
I don't want to betray my age but when I was working on my PhD more than 20 years ago. I did very primitive versions of the RAD on a chip and found I needed to use glass, Teflon and by time as much as possible in order to keep the test chemical in the medium instead of being sucked up into the system. That is a basic standpoint for anyone that is developing the symptoms is to be aware it is chemically resistant culture system, they're going to be more broadly applicable to test chemicals in in order to have a larger domain of test applicability, to think about materials using but also make them more expensive. You can think of cheapo--screening systems that if they are problematic you can get away with but also more heavy duty system that would be more hospitable to certain chemicals.

Miyoung Yoon
We have been working with more [Inaudible] systems in the flow system because of the use of the pumping and tubing system , we found really nice [Inaudible] from the system and it was about [Inaudible] and we relieving the compound. And [Inaudible] and it is really [Inaudible] a system in a way that we did not expect. So I agree with [Inaudible] about using more prioritization approach based on the chemical properties and based on the chemical space than we can determine whether we need to be more careful or force some groups we can be more comfortable of not defining those factors carefully at least for the screening process.

Richard A. Becker
It seems to me and I think this is a suggestion to get additional comment or reflection, that does the consideration that you need to do a print before you start doing the analysis and bio activity measurements because if it is, if you don't know what will happen, if you put a volatile chemical

into [Inaudible] and it partitions, you get a result but that result is not necessarily interoperable with the activities I'd let alone the kinetic side. It would be helpful that the input from those symmetry kinetics into the aspects of evaluation of bio activities is an important component to consider as well. So that is a question, do you agree?

Lisa M. Sweeny
More data is always better.

Richard A. Becker
Before you start saying I will test these chemicals, think about how you will interpret the data from a kinetic standpoint and will help you understand, these results will they be meaningful as well?

Weihsueh Chiu
This has been helpful in taking about in the standpoint of study design and bio kinetic measurements. You can think about what is the region of tests concentrations that would be interest or relevance in comparison to animal studies or human exposure levels that they are during the testing in.

Richard A. Becker
Any additional thoughts on human tissue slices from particular sources and how that could be potentially used to improve IVIVE?
I think, we probably answered it that you can now have the sustained organ culture systems that work well and I think the other, I keep reading about induced [Inaudible] stem cells and you can maintain functionality and those tend to proliferation with those cell types and away from tissue slice or human primary culture or culture from human tissue.

Miyoung Yoon
And want to add that we have not discussed much today [Inaudible] concern about what about [Inaudible] exposure and effect because of the repeated exposure if that can change both system and outcome and [Inaudible] and maintaining the relationship with the interaction with other tissue types that could help us to address more repeated dose conditions based on *in vitro* system. That is another area that we want to move completely to the *in vitro* base approach.

Richard A. Becker
Other questions in the audience? Let me try and rephrase this one. We talked about IVIVE and this is a question more traditional [Inaudible] kinetics but we can rephrase it in terms of IVIVE and asked the ocean, if you do these types of studies *in vitro* [Inaudible] and can you actually relate that not only to oral dose but in exposure that occurs by inhalation? And two models permit that?

Miyoung Yoon
I am so glad that [Inaudible] we show [Inaudible] approach which is IVIVE prediction used for [Indiscernible - accent speaker] and now [Inaudible] in collaboration with [Inaudible] and [Inaudible] level for use both in inhalation exposure and we have described [Inaudible] that [Inaudible] concentration for order daily exposure as well as inhalation exposure.

Richard A. Becker
I think the question is relating a 28 day study Internet but it sounds to me that the IVIVE approach can go to whatever route of exposure occurs because you felt the base model as component of that.

Lisa M. Sweeny
I will expand a little bit on the answer. Whether to use the oral status data, this also suggest possibly something that can be explored through and *in vitro* approach would be whether there is special susceptibility of lung tissue versus other tissue and something that can be explored *in vitro.* With inhalation exposure you have dosimetry to lung tissue than when you have the first pass effect and they can suggest limited *in vitro* study focused on the long or also shorter-term *in vivo* study. That might indicate whether you are likely to miss something rather than doing another study of the same length by the inhalation route and maybe replace it with a shorter study to identify whether it is a target organ of consideration. Not IVIVE, but to answer the question and add consideration.

Audience Question
Have a question, it is something I think about and don't know the answer and maybe you can help. How is all of this modeling going to deal with something that in the finding is increasingly important in all of these things? How can you model a debt microbiome into the cellular approach?

Lisa M. Sweeny
That would be another compartment in the human on a chip?
There is variability as much and more so than perhaps enter individuals and differences in [Inaudible] metabolism and another sort of variability but I don't see any reason you could not reconstitute another pre-exposure or pre-systemic exposure compartment with a gut content.

Weihsueh Chiu
From a kinetic point of view only if something in the gut that is metabolism horse transforming the compound that it will matter. And from a dynamic point of view, it is if a compound affecting the view, [Inaudible] and a whole different issue .

Nynke Kramer
Gets more complicated when [Indiscernible - accent speaker] and before trying to limit toxicity at my feet a problem as well I don't know *in vitro* there are a couple complex [Inaudible] models where we can put in bacteria and check the kinetics and uptake across the barrier.

Weihsueh Chiu
There is interest in the gut on a chip to populate it with microbiota but not quite there yet.

Nynke Kramer
These are humans as I'm talking about but anything but a microchip.

Richard A. Becker
Other comments or questions?

Audience Question
The question is about different population next February population next February 2 different population of females in different age. Other ways the IVIVE can be used for that extrapolation?

Miyoung Yoon
I say it is more of a high tier approach because we need to use PK models dealing with different life stages. There are *in vitro* studies available and the changes in metabolic enzymes activities during pregnancy in the mom [Inaudible] and [Inaudible] promoters we can include in those PBPK models we can describe the changes in kinetics in the mom as well as what is the compound. The compounds [Inaudible] and what is the compound movement and reaching of order internal exposure.

Richard A. Becker
The other is more of a comment and I will read it and we can discuss it quickly because of time. The question is sometimes screening is not as useful for particular context then more of a risk based approach; but what are the challenges now that one would face and we touched about it actually within AOP pathway framework of connecting. If you would the kinetics within an AOP where you are going from one key event, which is a dynamic event to another key event that linked by kinetic of the processes or exposures. What are the challenges in doing that getting to where you are predicting an adverse outcome? Separate from what we talked about today, patching things up and doing margin of exposure for priority setting and those are two different decision context. What are the key challenges you see in connecting the IVIVE or kinetic models to AOP?

Nynke Kramer
I would say return to look at the scenario and love as well. To actually develop AOP and quantitative AOP and rate of what level parameter [Inaudible] this event does it lead to the next event. To do that you need [Inaudible] and you need the complete very accurate identification of the [Inaudible] in these and something [Inaudible] and overtime, environmental science. We have TKTD models in the *in vitro* system to quantify the step, and the next step the next challenge. I see is *in vitro* we can test this and [Inaudible] preservation and link it to an adverse outcome and the whole animal and cause a relationship and also quantify; it and hope to look at the simpler model such as the elegance to do exactly this. But for a human it will be difficult.

Richard A. Becker
Significant challenges but that is why we do research.
Thank you.
We are almost out of time. We have some closing words and then think our speakers.

Allen Rudman
My name is Alan and I'm the FDA lead for this series and first I would like to thank Dr. Becker and Sweeney on the rest of the speakers for a great job in providing an overview and clarification on this complex area. It is a challenge to put this together in a great job.
Second, I would like to invite those FDA staff who want to have lunch with the speakers, we will meet in room 2A023 and about 10 to 15 minutes. And the speakers if they would meet at the registration desk, we will escort you to the room. Third, the SOT has agreed to put the webinar link and information about the CCT meeting tomorrow on their website and it will be on the SOT website under upcoming meetings and if you look under December 2 it will be there. Again from 10 AM to 2 PM. Finally I would like to thank Betty Eidemiller and her wonderful staff, for the great job. She has done this for 2 1/2 years and through rain and snow we have had these, wonderfully. Thank you Betty.

Finally, there is a colloquium upcoming at the end of March, and maybe the last week in March. The title is “Clarifying *Adversity* in Food Safety.” This will be an interesting one, covers a number of areas. Finally in May and June we will have colloquia; the titles are still being discussed.

Richard A. Becker
Let me thank the audience especially for your engagement and participation and those on the web, we appreciate it and reminder this is brought to you by the foresight of FDA and working with SOT and benefits not just one factor of the side of toxicology but globally and appreciative of that thank you --. Thank you for allowing us to have this colloquium.

[Event Concluded]