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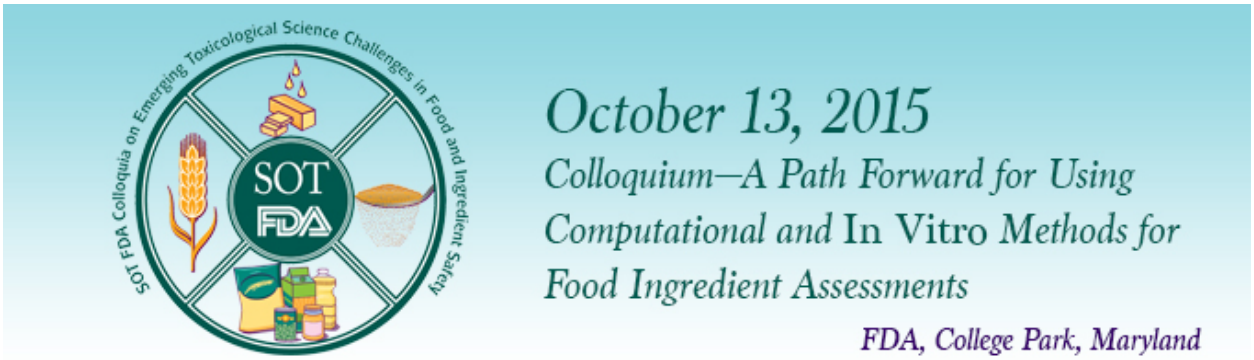

A Path Forward for Using Computational and *In Vitro* Methods for Food Ingredient Assessments

Schedule

8:30 AM–8:40 AM	**Welcome from FDA and Overview** Suzanne Fitzpatrick, US FDA/CFSAN, College Park, MD
8:40 AM–8:45 AM	**Welcome from SOT** Peter Goering, SOT President, US FDA, Silver Spring, MD
8:45 AM–9:10 AM	**Development of *In Silico* Tools at OFAS CFSAN FDA** Patra Volarath, US FDA/CFSAN, College Park, MD
9:10 AM-–9:55 AM	**Gaining Confidence in Replacing Animal Tests: A Case Study of the Endocrine Disruption Program at the US EPA** Richard Judson, US EPA/NCCT, Research Triangle Park, NC
9:55 AM–10:10 AM	**Break**
10:10 AM–10:55 AM	**Read-Across with Computational and *In Vitro* Data** Elisabet Berggren (by webinar), Joint Research Centre, European Commission, Ispra, Italy
10:55 AM–11:40 AM	**Use of Computational and *In Vitro* Data in Cancer Hazard Assessment of Data Rich Chemicals: Examples of IARC Monographs** Ivan Rusyn, Texas A&M University, College Station, TX
11:40 AM–12:40 PM	**Roundtable Discussion** Moderator: Ivan Rusyn All Speakers

Welcome from FDA and Overview
Suzanne Fitzpatrick, US FDA/CFSAN, College Park, MD

We may as well get started here today while we're waiting for people to come. My name is Susie Fitzpatrick and I have the honor of introducing the latest in our symposium, between the FDA and SOT to present some cutting-edge technology in the field of toxicology. We feel it is part of our mission to introduce some of these things to our regulators so that they start feeling comfortable with some of them different techniques and begin to incorporate them as part of their regulatory duties. We have a great lineup today and I will tell you all the important things. The bathrooms are right outside by the registration desk, there is coffee out there for anyone who wants it, there's a cafeteria around the corner outside the FDA where you checked in. Welcome to everyone, we have a lot of people online from all around the world and we are expecting a lot of people in the audience today. Without further ado, I will introduce a valuable FDA scientist, and the president Peter.

Welcome from SOT
Peter Goering, SOT President, US FDA, Silver Spring, MD

Thank you, Susie, on behalf of the society of toxicology, I would like to everyone here this morning in the room we have a considerable people number of people participating on the WebEx, and welcome to them as well. We have a considerable collaboration between the FDA and the Society of toxicology. These are issues related to toxicological challenges for food safety, and ingredient safety.

The SOT is in its first year of a new strategic plan. We are focusing on meeting the challenges of keeping toxicology at the forefront of scientific advances as we work to improve public health and environmental health. One of the priorities is to develop and support toxicologists which includes training. The basis of that is to train each other in the latest advances in risk assessment and other issues with food and ingredient safety. We are very pleased with our success in the first year of these colloquia. We had it up to an average of 300 participants here or on the WebEx and this was a great deal of success and participation that we would like to continue as we go into our second year. Last year we had four highly successful colloquia on various topics of PHOs which are partly hard to judge, partially hydrogenated oils and how to use ADME and a meter toxicology and risk assessment in food safety and cosmetic session assessment.

I want to make this of you aware, those of you aware that we have these colloquia available and recorded on the website. We've had a number of people download the videos and a very high number of individuals who downloaded the slide presentations for viewing. We are also pleased that we had a strong international participation seems to be growing throughout the sessions and we had members of toxicologists in 26 unique countries participating in the WebEx. This was a challenge for some of these individuals who are awake at all times of the evening in very early morning to participate. These are the countries where we have had participants on the WebEx. They are countries that have very rich toxicology activities going on and including those

in an emerging area for developing their toxicology expertise in the country. We always take surveys, we will ask you to do a survey at the end of this. These help our organizing committee to develop programming for the next year, here's an individual who participated last two in Australia and stayed up until 1:30 AM watching the webcast and found it very beneficial.

These sessions don't happen in a vacuum and I would like to take this opportunity to thank the organizing committee. These are individuals for the Society of toxicology as well as the FDA. I would also like to thank our speakers today for their participation and particularly the Society of Toxicology staff who helped put this together. Betty, the director of education is here today. There are three more of these scheduled for 2015 and 2016. On December 3, there is a colloquium with the topic of carcinogenesis, then in the March and April time frame more risk assessment, state-of-the-art professions of toxicological concern and finally in the April or May timeframe is a colloquium on sensitive subpopulations.

Today's colloquia are a path forward for using computational and *in vitro* methods for assessments. I look forward to the session today and with that I would like to introduce the chairperson of the session today, Dr. Ivan Rusyn from Texas A&M University. It is my privilege to share this and introduce the speakers today. As you have seen our last one, it was in June on contemporary issues in this could assessment. For that we focused on traditional risk assessment systematic review and mechanistic data and this response assessment. What we want to do today is look beyond the traditional risk assessment paradigm and considering how some of these data can be used to make decisions. We hope you will participate by asking questions especially those on the website, ask questions and at the discussion we will monitor your questions, so you are welcome to participate as well. I would like to open our program and we are very pleased to have a representative from the FDA, and she has her master's from Georgia State University and she transition from genetics and biochemistry and biophysical chemistry into more of the quantitative chemo informatics and was instrumental in the development of these when she did her post-doctoral fellowships there. She is now part of the EPA and the office of attitude safety and she will tell us about additive safety and she will tell us everything we need to know.

Development of In Silico Tools at OFAS CFSAN FDA
Patra Volarath, US FDA/CFSAN, College Park, MD

Good morning everyone. Those who are here in person and those who are on the phone, good morning. First of all, I would like to think the organizing committee for giving me an opportunity to talk to you about quality computational activities that we have going on within the office of food additive safety, let me see if I can find my slide. We are loading. I will first talk about the chemical evaluation and risk estimation system. It is one of our informatics efforts we have going on within the office then I will talk about two of our recent projects that we have started, the QSAR model development for food ingredients and evaluation of them feature data for applications to food ingredient assessment.

Just a little bit about the history of CERES, it was created for several purposes. One was to address the technical challenges that we have with food ingredient evaluation processes. This was located in different locations and had different data format. It was created to consolidate this information in one place and to bring everything standardized. It was designed also to be a knowledge base, chemicals regulated. The majority of compounds in CERES were our food chemicals that we obtained from the database and PAFA systems. We bring in compounds from other sources who stated we believe will be beneficial to all the viewers in the office, so we went ahead and created this place in the back end of CERES so once we understand how to use these data sets we can quickly bring them into CERES and back in. It was also designed to provide capabilities, so our reviewers can come to CERES and only to look up information but to use the building terms of we provided to interact and analyze data within CERES. First version 1.0 came out in 2012, the second one came out in 2014 and the latest version has just been released back in August. As I mentioned earlier, one of our data sources for CERES is the PAFA database that have been regulated since 1970. The information contained within PAFA is chemical information, and toxicology information and regulatory information. We started updating it sometime back in 2010 but because it contains such useful information on these food additives, we decided to bring the PAFA data into CERES. Another set of information is the FAERS system. There is other data relative to supporting the sit with you. We do not absolve everything, we only extract information like chemical information, regulatory and toxicology information into CERES, just enough information so that our reviewers would know if we had seen the substances before and if we had, we would know.

The majority of our studies came from the PAFA database because we import everything from PAFA into CERES. We have studies that our interns harvested from the original studies and with CERES to point out we have the 2.0 we have this. From all the studies that we have, the categorize them into 32 assays -- assays and put them into CERES. In terms of chemical information in CERES, we have a little bit less than 18 it doesn't compound that contain various types of chemical information. Chemical identifiers for example, every single compound has a unique CERES ID and may also have chemical names or identifiers and if we put them in from other sources they have the original identifiers as well because down the road would like to cross-reference our compounds of compounds in of the databases and use those identifiers. We also keep track of where we got our chemical information. The majority of the chemical names and cast members we obtain from our own database. For food compounds we maintain values function so that all of you would know what types of chemicals they are looking at and if applicable, we will link those compounds to those cumulative estimated daily intake values from our database. For those that have structures, we obtain all structures from trusted sources like SciFinder, the chemical abstracts service, and collaboration with DSSTOX and Altamira. the chemical data in CERES also expect to toxicity and regulatory data also links to that.

This is how they look when they appear on CERES, so as you can see here some of the chemical information that I mentioned is being displayed right here along with the structure of this information and other identifiers and chemical computations can be

found by expanding these times. The regulatory information, chemical information and toxic information are organized in the same fashion. If you expand this ID, this is what you're going to see. They will all be listed under the source and you can get down to the lowest level of the assays to see the study and test data. In terms of use interaction and source capability, users can enter a compound into CERES by using different options. Into the compound by chemical names, identifiers and cast members and they can couple the chemical surge with the toxicity search functions on the right. It can also structure to series by drawing the structure so if they go into sketch molecule tab, another window will pop up. This is where the user can draw in the structure by using the drawing tools that we have available to help facilitate the drawing. We also have a few informatics workflows available. 2.0 we activated to workflows, one is toxicity where they can go in and play around with the QSAR models that we have. They can go into export structures and data is to export data on selected compounds. They can also go into workflows to compare structure similarities. This can probably threshold of toxicological concern calculations with of evidence in decisions and these workflows will be become available in the future.

Where are we with the CERES project? We have been working to try to get things up to speed and find the data architecture in the back end in the user enter graphic. We are at the point where we can boot the system up to another level because we're working with those teams to create a system called TRAM that will enable communication to produce these three systems to take place and communicate automatically in real-time. They will say now have information in our database, so you can grab it. We are working on designing information tools and my review memo tools for interns and reviewers to go and put in their assessment. Once that information is approved, it will be deposited to the destination places automatically. This is the focus of our current development for upcoming releases and in CERES as well as the other systems.

The model development for food ingredients, QSAR quantitative structure activity relationships which is an analytical application that can be used to the chemical toxicity, is on chemical properties and structural features. Our reviewers use models to predict toxicity of compounds that have very little or no toxicity data available at all. Sometimes what the notifier's use to match what our models predict. One limitation with this approach is that the predictions coming out from these models are only as accurate as the data that went into the training set. The information in the box is vague or wrong, then the predictions coming up would not be as accurate as you would expect to be. That is one challenge we're facing when we use the commercial model that come with the software we purchase is that they may be too generic. They may have been optimized for the compounds that each company uses to build the models that they may not be sufficient for the type of compounds that we get to coming into our office for review. What we need are QSAR models that have been specifically designed for our food ingredients. In CERES 2.0 we have eight models available. They have been validated by collaborators. We are in the process of testing these models to see how applicable they are to the food ingredients that we have. We went into food ingredient databases and we took chemical names, extracted out their structures and send the structures through the models. For each of the models we want to see how many of

those structures fall with the applicability domain and how many falls outside the applicability domain. Those are compounds that are models have seen it examples before and for those that are outside the applicability domain those are the compounds that our model has not seen of examples before. These are the compounds that we are interested in because by analyzing the chemical structure, we can derive a new set of rules that can be added to the model so once we do that we can recycle out of these two optimize the model so that the applicability domain will be expanded to cover the out of domain compound. The advantage of these approaches that it provides are better predictive models for food ingredients as well as transparency so that all the viewers will have better transparency and models to do these predictions.

These are the results from the first round of sending those structures to the model. The majority of the compounds in the public databases fall within the applicability domain for all the models and we do have some outside the applicability domain. We're looking into these right now, to figure out what is it that our models don't know so once we add that factor into the models, we can recycle these compounds back into the workflow to optimize the models. We submitted an abstract for this work and are planning to present the results in more details on these projects including validation and performance evaluation on the SAT next year.

I would like to spend the last few minutes of my time talking about another project we are working on, it is evaluation of *in vitro* data for food ingredients. It is ToxCast face to that we talked about in December 2013. Included chemical structures and information for compounds, along with the structures that contain over 1000 *in vivo* toxicity outcomes and over 700 high throughput results for the structures. To get a better understanding of the biological activities, we went into the literature extraction of the descriptions and put the number of assays right next to them. Would like to be up to do is determine the relationships between traditional toxicity ToxCast. For these we used the ones in CERES and compare them to the high throughput *in vitro* assays and to bring that knowledge. We looked into chemical coverage how many of the ToxCast and Tox21 and for those of you who are not familiar with these programs, ToxCast is a chemical prioritization program initiated by the EPA. The chemicals in ToxCast are also in Tox21 which is a larger collaborative research project. For the work that we do right now we focus on ToxCast because we don't have access to Tox21 assays yet. Once we get that we can start applying the analysis to the data set. Going back to ToxCast, we took these 907 overlapped compounds, went into the ToxCast assay data and mapped those onto the assays in CERES. The Y axis on this heat map is the CERES assay. All of the assays contain overlap and the chemicals are being clustered in this region right here. Now that we have identified what these assays are, here in the process of evaluating the relationship between the ToxCast assays and the CERES assays and try to figure out how we can use ToxCast assays to complement what we have in CERES.

This is our progress in each of the projects. They continue to harvest more data to enhance our database and we are expanding our chemical library to cover chemicals of interest and we are building in more informatics capabilities within the system. For QSAR model development we are in the process of optimizing our models for food

ingredients and it's going to be a continuous process as we add more chemicals into CERES to make sure our models cover all the compounds. We looked at *in vitro* data sets and identified which of the assays we need to focus on and now we are evaluating relationships and we will bring that knowledge into CERES to make available to everyone in the office. For more information, right now the system is open only to staff, so those of you who are here online who would like to have access to CERES, please contact our team lead and these are two of the publications we have available for those systems. Last but not least, I would like to acknowledge people who contributed to the project. That is pretty much everyone in OFAS. Everyone from the clinic works teams, my colleague Leighna is leaving the data harvesting team and everyone else put so much time into curating and putting time into CERES, thank you.

Audience Question: Between three and CERES, can you elaborate on how you are using ToxCast and Tox21 data? Are you thinking of building models that take some of these combined *in vitro* endpoints and QSAR models, if you could elaborate in this last point in your summary?

Volarath: For now, I think we're going to try to look for relationships between ToxCast assays and what we have in CERES just to understand if we can make that direct link. For those that don't, we can start asking questions. What is it about these assays that we can make the right connections and then we will go on from there.

Audience Question: Very interesting presentation. I have a question on the CERES worksheet, there was one element that said the study quality. How much of the data information is quality assessed? When you opened up one of the expanded elements of the study sheet, it said quality of the study not determined.

Volarath: We extract the studies directly from the submissions, so the qualities of the studies have been curated by our toxicologists that have done their assessments.

Audience Question: That is not reflected in your summary sheet. There's no way to tell whether the data is good quality, then quality, whether it meets GMP standards.

Audience Question: Since we started updating the PAFA database a while ago, whatever was the original that was in the PAFA study, whatever curation that took place before that, we assume that is the standard…That must be something to look at, I'm sure that's going to be, I know it has been in my mind and will be an issue.

Gaining Confidence in Replacing Animal Tests: A Case Study of the Endocrine Disruption Program at the US EPA
Richard Judson, US EPA/NCCT, Research Triangle Park, NC

Rusyn: Our next speaker is Richard Judson, who has been at EPA for almost 10 years now. He is a chemist by training, got his bachelor's and Bryce and his masters at Princeton then had careers with bioinformatics and pharmaceutical small and medium-size companies in coordinating their bioinformatics activities before joining EPA, is that correct? He has really been on the forefront of not only getting the data but actually working with stakeholders and pushing it out into the domain of use, so we are really pleased to have Richard come talk to us of the most recent success stories.

Thank you to the other organizers. I'm going to go back here, notice here at the bottom, I may editorialize. This is the Environmental Protection Agency. The group wanted me to come and talk about a success story using alternative *in vitro* assays for solving a real-world toxicology problem, so you can decide for yourself how this is. It has to do with the endocrine disruptor system. For this up to who are in that field I want to set the stage for talking about a particular case which was Diethylstilbesterol which was developed to hopefully prevent pregnancy loss and competitions. It was used for about 30 years, P attention because some of the story has to do with how long it takes for things to happen. What happened was in the early 70s, young women in their late teens and 20s who had been exposed in utero for a few days or weeks started developing some rare reproductive tract cancers and infertility and it was traced back to having been exposed to DES. What this does is sets up an idea that certain chemicals are almost like timebombs. You plant them and 20 or 30 years later you have this problem. So that's one of the features of worrying about endocrine disruptors, they can have a long-time lag and are getting into the developing embryo and early life stages of children and having these effects much later on.

This is kind of a bad probably need to worry about but the consequence of this phenomena being discovered, Congress passed several updates to laws in 1996, women started being doused in 1940 and resolve the problem in 1970s, and lockets passed in 1996 and they added provisions to the pesticide regulations which is the drinking water regulation, it required all pesticide ingredients but all chemicals to reach significant populations could be exposed through drinking water what had to be tested for their potential to the endocrine disruptors. The DES example which is a synthetic estrogen, the regulations really focused on estrogen mimics. I couldn't tell you what this stands for, many government acronyms. It got expanded to thyroid pathways. That actually moved pretty fast. There was a recommendation to set up a screening panel. And over a period of years, you can see some of the dates here, in 1998 we needed to have this screening panel worked out. It took until 2009, it was put together into tears and sort of the talks world or the endocrine world, screening means past. You quickly go through and say which chemical have the potential to be hazardous? Those you can test that you can do response and risk assessment. This set of screening assays were put together and the issue test orders for 73 chemicals. In 2009 it took three years of legal challenges NCR owners ready to start testing and EPA got results for 52

chemicals and just now are being released. That is about 19 years from the time Congress passed the regulation until any data is really finalized. Evasive what's the difference between 73 and 52 chemicals? They are so onerous. A number of companies said our chemical is not valuable enough to spend that money on, so they just withdrew chemicals from the market. From a health standpoint maybe, that is the right thing to do although that was not the intention of the regulation. That is tier 1, tier 2 and a test orders have been issued today I believe. What is tier 1? It comes from the screening program website, so you can see five *in vitro* assays, two of estrogen, one for androgen and these are the pathways that actually create estrogen and androgens. There are six *in vivo* assays and I'm pointing out that trans-activation and you to tropic, that's really what I'm going to focus today. A serious of more complicated assays. My success stories going to say to the point where we can replace the first study but that is by far the simplest. The metamorphosis and short-term reproductive studies are the ones that are the most onerous, expensive and time-consuming so we are still fairways from that.

This is the current panel as it exists. The issues I've already mentioned, one problem is the cost, it's about $1 million per chemical and out of people who can run these tests there are only 50 chemicals a year. It's once the text group got involved with this six years ago that everybody said will how many chemicals are there? So, we built big databases and were able to tell the endocrine program, there are 10,000 chemicals there. So suddenly we had many billion dollars of backlog and would take 200 years to get through this. To get to the quick part of it. Clearly there had to be some other approach and that was when competition toxicology approaches. Initially we were asked, can you just prioritize? In many lifetimes we will not be able to get all of those that can we test the ones that look the worst first? Then we got moving along and saw that some of these approaches seem to be promising. This is the origin of that program and Vicki was the lead in the pesticides program. She was hired recently but was a key driver in some of this.

I want to throw some dates out of here, this was started in 2007 about the same time that it was parallel and the toxicity testing of the 21st century report, that organization must be organized and thought through about the same time independently. It was nice when the report came out to do some things that we were already actually making good progress on. We were screening about 10,000 compounds to the Tox21 library and those 3000 would be went to the endocrine related assays. There is a big fraction of the compounds that we will have from the data I'm going to tell you about. We have a lot of model organism data and it is really fascinating stuff to see. And EDSP21 is the ones we're looking about.

How do we think about replacing *in vivo* studies are doing toxicology little bit? We like to think about not only just hazard risk and exposure, so we have a big hazard piece that I will tell you a little bit about and a large exposure component. In our center, the competition toxicology Center, the basic idea is that if you can estimate hazard and estimate exposure and there's not much of an overlap, maybe that is important chemical and that there is no anticipated exposure scenario or those overlap, let's but that is a

low priority and worry about where there is significant overlap. The other key idea is this idea of uncertainty. Both our hazard and exposure estimates are uncertain and the further you get away from animals, the more uncertain they are. Even within that uncertainty we find there are lots of chemicals where we may not know the exposure within two or three orders of magnitude that there is still a great big gap.

The focus is this estrogen receptor model, let's take a lot of different assays and combine them together in a weighted evidence model to say that this chemical can interact with the receptor, so it is a potential endocrine disruptor, or it can't. It's kind of an adverse pathway but it doesn't have all of the flavors there. A key point that we discovered from all of our work with the ToxCast data that you say mine gives separated as if you disagree with that you're right. Every time someone comes to us and choose as their data we find out that for these chemicals you're getting the wrong answer for reasons we understand. That's why we have to integrate different assays to get rid of some of the noise or average that out.

In order to the comfortable with the model, what we've developed as a pretty generic process for modeling that data. One of the reasons we have to worry about getting the brunt answer is because all of the in vitro assays were developed from pharmaceutical chemicals. It's a couple Katie piece of space but it relatively small piece of the entire space and especially when you look at the types of things that the EPA looks at or the FDA, we have solvents. A pharmaceutical company would never test their solvents, just metals and inorganics and these things are really nasty. If you have solvents or surfactants, they can actually do need to the protein which in certain cases can give you a beautiful binding signal but has nothing to do with binding. You can dissolve cell membranes. So, you need to take that into account.

This heat map, this is all the chemicals that hit at least one of the assays and these are all the assays. Industry when it was estrogen, but you have this were all of them would turn on and you would have all the stuff 10 here. Were as you have a couple that no others turn on. These assays are somewhat metabolically competent. There is some evidence and we're working to try to understand that that some of the chemicals that have both of these are ones that are being metabolically activated to be estrogens. Let me teach you a little bit of scepter biology, I couldn't show any equations some at least going to show some biology here. This is an artist rendition of the signaling pathway and for the moment focus on these errors. This is the actual receptor chemical binds, two copies come together and then move into the nucleus and recruit cofactors to create a transcription factor. It sits on the DNA and DNA makes RNA and RNA makes pretty and triggers the estrogen receptor, you get so proliferation and certain types of cells. That's kind of the real biology and we can go on an incredible voyage and we can actually watch all of that happening. Of course, we can't. We have to look at the cells from outside, which is why use assays. They give us an approximation and that is what all of these are. We have three that just measure binding, six assays that measure DNA-binding, RNA transcription, to the measure protein production and one that measures and proliferation. This is the agonist and antagonist mode, if you have two bindings, this is the heat map and these last two are antagonists whereas if you have some process,

only these three will turn on but since you don't have real activity it won't turn on the others. We developed these receptors, mathematically we can distinguish between the pseudo-scepter to turn on and that's the basis of the model. But anyways, what the model does is integrate the 16 agonist assays and comes up with a final score. It's really just a summary of the potency of all of those and we have a large series of reference compounds which actually came out of international groups to define those and they define not only should the compound be positive or negative, but these are ones that are supposed to be positive and they are so that potency goes from left to right except for a couple, we don't see any activity in these because we only test up to about 100 micromolars. These are week compounds so the only time you see those it's when you go up to 1000 witches beyond. We do a pretty good job of distinguishing positives from negatives and this is the potency class at the experts should get, we get a nice quantitative ranking.

So now we can take lots of compounds and the publication is 1800 compounds. The standard screening can do 50 a year. We've done 1800 and couple of years and have another 1000 going through right now. We feel like we can definitely tell you the right answer pretty well for *in vitro* pending. The real goal is can we start replacing *in vivo*? The idea is, can we take this collection of cell-based assays and compare them to the utero tropic assays but that we are trying to do is figure out human relatives and this is the model we are using, let's see how well those compare. What they did was to take the chemicals and do it literature survey for guidelines. How do you know the quality of the study? There is a very expensive literature generation effort, so they came with 700 papers and numbers of descriptors of the study and you had to meet a certain minimum criterion to be included. Finally, they were able to come up with 98 chemicals with 442 guidelines. So, they didn't necessarily follow the guidelines executive it was close enough. What they came up with finally was 43 chemicals that had more than one guideline like study. I think I will have this on the next slide, we have these 43 chemicals. Notice this is an assay has been run for decades so there are not that many chemicals that have been run more than once. That says something about throughput. I'm not going to be to all of this, this is a minimum criterion that a study would be considered guideline-like. There was a lot of work done on immature mice and it was felt like the experts said those studies were to sensitive. You can get access to the slides and look at those.

In order to compare with the estrogen receptor model, we took those studies, the 43 and 50-yard chemicals that have been run, they were brand-new and had been run and the feeling was, they were under enough scrutiny they were probably pretty good. That God is out to 41 chemicals and 31 in active. There's my punchline, what I'm showing is the rank order, but this is the model score from the *in vitro* model and if the *in vivo* study said negative and it's clear blue this a positive. This kind of a cut point if the score was above .1, all the positive accept this one which is cleared rapidly which is why it is not *in vivo* positive, that these are the ones we worried about. It's a silicon oxygen compound and I talked to the person who in the studies and they actually ran them twice independently because it was such a strange looking compound but also extremely volatile. There was a good chance when we went to run these it was evaporated and

was never in the well. We are not too worried about that, but other people could be worried about that. Very high accuracy comparing *in vitro* to *in vivo*. Here was an interesting problem that the folks discovered, this was guideline like data, the highest quality. They found out that the chemicals were run at least twice, a quarter had one lapse in the chemicals positive and once a negative, so there is a high discordance rate and partly because the protocol is set up, so it is not really powered to see very weak affects. You only had to have five or six in the positive group in there are all sorts of covariance that you don't necessarily control for. We can predict the study, and this is the most extreme example, somebody when up and said it was negative, most of the negatives they just did not tested very high, so this is a protocol issue.

So, given all of that, the EPA put out a Federal Register notice and I wanted to read a couple of key points, essentially that we are now ready to accept the results of the model in lieu of *in vivo* and this was an interesting point. This incorporates and validated assays. Another problem is the validation process so that even the estrogen receptor assays that were part of 21 and took years to validate and we said we ran lots of chemicals, lots of reference compounds so these are as validated as anyone out there, they can serve not as a prioritization tool but as an alternative in these methods can accelerate the pace of screening which was supposed to be fast does not so we are going to decrease costs and reduce animal testing. The Federal Register notice, he put this out there and request comments. I don't really have time to go into that but maybe we can talk about that later. It was everything from this is great to this is horrible. It's always kind of like that. One of the things we find that it's important to do is make everything transparent and make sure anyone can see the data and use it, so we put together these dashboards you can see behind our decision. We put together a consortium of about 20 or 30 groups around the world to build QSAR models, so we can project that into a much bigger place. One of the classes of comments that we got was there are some industry comments that said we can't see all of this and it was partly because the regulators got a little bit ahead of the science and put the register notice out before the paper was published but we will send them all of the data and software behind this so it's very transparent.

In summary, this estrogen receptor model is a first example of success because as far as the regulators are concerned, we can start accepting non-animal data to replace some of this animal data. This required a big collaboration between people doing validation and the next step is where we are doing the same thing for the androgen receptor. We are hoping to get a manuscript out, and this is the equivalent and we are looking at getting that database built up. We have about 1000 chemicals and a lot of work going on in thyroid which is a more complicated beasts. There's not just one receptors. I will come back to this in a second and these are the specific people involved in the estrogen program. I'm bleeding the office of research and development and we have done all your trip data, you can see the key publications that are outside your trip database and I'm not sure you can see it today but if you were to write to me you can get a copy of paper that came out. These comments make some interesting reading, so thank you very much.

Audience Question: Thank you Richard. I will start questions on some of the technical notes. The most difficult part of the modeling was not necessarily running the assays and building the model, it was finding the right *in vivo* set to compare against. How much of the comments that you got to the Federal Register notice had to deal with the fact that you are comparing a very small set of *in vivo* for those standards? To follow up on that for the subsequent process that you are planning are well underway, what is your estimate of the standard against which you will compare for the *in vivo* part?

Judson: We got no comments about the size of the *in vivo* data set and if someone had, it would just open up the issue that almost no assays, is there very much consistent high-quality *in vivo* that the validated against? This whole idea is coming from a big data side is available troubling. I don't know that we are doing the same kind of thing for these others, people are scouring the literature, calling friends say what kind of data can we get. One point that I think is important moving from *in vitro* to *in vivo*, the problem with *in vivo* gives you more information about whole organism effects. I just lost my train of thought. Other data sets, okay sorry, senior moment here. It takes a long time to develop the databases. With the *in vitro* methods, there are no it's coming along monthly and we run all of these the do not have metabolic competency with maybe a couple of exceptions that but you can run 1000 compounds or 10,000 compounds so as we develop assays that run in stem cells are some new kind of an point, we can turn around tomorrow and within a few months have 10,000 compounds run. We have this consistently improving process that the methods are amenable to their *in vitro* is not really able to do that.

Audience Question: Because new assays are coming up and are hopefully better and better, your accuracy is already close to one, so do you need better assays? And you close the book and say yes, we will come back to this but right now there are other problems for us to solve and we're moving on to other things? Are their thoughts to that expects?

Judson: We need it for two reasons. Typical reference chemicals, people, and they are really positive and negative, or the structures look really positive. This is kind of an easy birth to me. We know there are compounds that are not like these. One of the big criticisms if you look at the Federal Register notice, everybody but PETA said we know there are other ways that you can get this, so you need to solve that problem. We were at a meeting last week and we will talk about this later on, the idea that you cannot start replacing *in vivo* with *in vitro* into you understand metabolism, it's not going to go away until we solve that problem. Using estrogen as a testbed for this is a good place to start.

Read-Across with Computational and *in vitro* Data
Elisabet Berggren (by webinar), Joint Research Centre, European Commission, Ispra, Italy

Rusyn: Thank you for coming back. Coming back prompting finish your conversations. Our next speaker is contest remotely so we're excited that Dr. Elisabet Berggren agreed to participate and we believe we have her on the line, let me give a little introduction. She is with the joint research center systems toxicology unit in Italy and she has been very much involved with read-across and different stages and organizations in Europe first with the European chemicals agency and then the joint research center. She got her PhD from Stockholm University in physical chemistry and has been in various organizations in Europe since then. The group that organized this workshop, we thought we would be remiss without bringing something that the subject of conversation with Europe. Think this is coming here as well so we're looking forward to your presentation on read-across with competition of data, thank you.

Thank you and good morning to everyone, I regret that I'm not with you and can't see you because it's always nice you to see who you're speaking to, but I see we have a lot of participants through the web. I will talk about read-across and how it can be supported by competition and computational data. I will say that I can speak about this research and that is supported economically from the European Community. From the program, as well as on the cosmetics union.

What is read-across? It's when you have robust data set for that you call them the source. You actually take all of the data sets and you can read it across the data substance, the target. That is not similar enough to use the same data sets for certain endpoint for a risk assessment. For example, if you have a 90-day study for the source substance and you have convincing arguments, enough to say that your target substance is similar enough to the source substance to take the full study and uses the same mineral value for the target as for the source. You can do this in an analog approach give her to stop themselves to substances and these transformation results in the same substance that is actually the toxic substance are you have chemicals brought, you assume that they are acting on the body in the same way, so you have the same sort of interaction and the same adverse effects. Then you can have a category approach and it's a little bit different, normally the structurally related substances, you can see a trend in this substance and you can read-across especially if you have a substance sort of in the middle of these things you can predict how the toxicity of the substance will be. Even the next case where you have a plan, you can have a mechanistic explanation for what is happening and you are less concerned about your with across, you can have a trend in other properties you decide you can carry across, this is less convincing but sometimes it is needed because you may have a substance that has low toxic effects or no toxic effects and then you would be able to read-across to an unknown compound. You need to in some way show that it is not biologically active.

Why don't we just test everything? It's good to avoid additional animal testing to save time and cost and sometimes it's that we have human data before a certain substance or several compounds in a category and that it can be used to be the cross -- data if you have data on a pharmaceutical, that can be read-across but is perhaps less relevant for the risk assessment substance. Then of course you have the possibility to -- safety assessments and that is of course useful to make it more efficient to have a safe environment of chemical surrounders. If you do read-across I structural basis only, I have robust data in the middle here but you can see that it's different depending on the carbon change length and then I can say quite confidently that a certain endpoint is getting more toxic or less toxic a but also this is not the case of course because you usually have a little bit of data on different compounds in the same category and you don't really know how to do this. You would like to have biological similarities, I can even be that they are more important because perhaps you are handling the substance but there's actually a mixture of different compounds and you need to try to read-across complex carbohydrates and that's more important the biological similarities in the chemical similarity. Biological similarities can be based on how these active group of these chemicals are interacting with some enzyme protein are you have *in vitro* data that you can use. Biological similarities of course can provide confidence in the chemical structure and I think this is something that we are looking for to make it better and more confident read-across. You can see here you can look at the way to illustrate this. This is a biological read-across approach, so you can see that smaller thoughts on lines are the chemical similarities the black ones, so it can give you quite a good flavor of how different compounds are similar both chemically and biologically to what you are looking at. Ivan said that we have more read-across and I assume that it is because we're collecting a lot of data and risk assessment are chemicals especially in that it has been useful. Including this reader cross categorization, the highest rate read-across is… This is not to say that one of these predictions would satisfy regulatory requirements. It is my feeling that most of them don’t, but it shows the need for these types of assessment of chemicals and is very useful. Toxicity testing is expensive and tedious perhaps there are not that many concerns. That is why we need to look more into read-across and make it better. This agency has published guidance and I will also mention later that they have done these assessments which is a little bit complex is a first look, but it is really to guide the register through the mechanisms to read-across one chemical to another.

This is an example from the European legislation implementing the globally harmonized system. This is not a structural with across, but this is actually the solubility which are very important as well. For example, we have five compounds that have been risk assessed within the legal framework and based on this data, it was 118 compounds depending on that solubility. Anything that had to be done was responsible for the effects to be assessed. We had a few cases where we had to look at and of course also to understand how large concentration could be on the site of action so that was a determining factor of course. That is related to the variability and in this case, we assumed that water solubility and compounds would be available at the site of action and that made legal implementation of a lot of compounds forcible.

This is an example quite difficult read-across and that was discussed on the biocides legislation. These are the anticoagulants. This is a very particular case because you had one of the enter vitamin K substances that is used as a pharmaceutical and it has been used as a long time as a medicine to make it more soluble. This is the same effect because actually the rats are killed and bleeding. They are similar, and it is also related to business that this group is very difficult to test and usual animal experiments. So, these would then be used with emphasize.

The concern is that the reproductive toxicity because of course you have this effect. It's known to be related to toxicant and the others are not proven to be so. At the same time there is no evidence that it could not cross the placenta. For the other anticoagulants that were tested for the developmental toxicity, it was shown that there were actually know signs of developmental toxicity. However, there was a lot of discussion where actually the baby, the developmental studies could be reliable and here they have the case that they are structurally similar. We have human data in a very large database of human data, but you could never know what the outcome of these tests would be. Due to the negative data they agreed that the source was not accepted between Warfain and the other compounds. It would be helpful to have additional data to support the read-across in the substance category.

When we improved the read-across strategies, sometimes it is actually, the traditional read-across is not good enough that these sources are very helpful way for the existing animal and human data. Then use the read-across tested chemicals. You can of course see that there is a lot of approaches you can use. You can have *in vitro*, you can have computational, QSAR is, and if we get it right and try to make it more sort of structured, how to do a read-across. It will make it easier to accept it. You can think about that, you can replace an animal study with a nonanimal alternative method and you are sort of saying that your read-across and you have predicted the effect of the target substance. You can also use the data to read-across, so you would example confirm the similarity in the mechanistic actions. You can also say that they might have the same effect. By some additional evidence, you can actually say something.

We are in one of these very large European projects and the third one is research that was started five years ago and it's to go placement of toxicity testing. Of course, this is the area that is most difficult to find replacements for animal testing. It's a collaborative project and they are all sort of expertise in different states of *in vitro* assays, so the different projects are sort of, mentor to each other and it's a very economically large project in the beginning of the presentation like I said between the European commission and the cosmetics world. We are spread out in 16 countries and you can go and visit the homepage and find the annual report that we have for each year of activities. You can find out the information as well. These messy for team and health and to use this knowledge to develop gravitational and experimental models that predict points of departure needed for safety assessments. Is not always looking at alternative methods, but it is aiming to take be toxicity that these predictions can be used for safety assessment. You look at molecules and initiating events, and before that you have to have some sort of listening about the compound. Then you look at different key events

from those in the molecule events you can predict the outcome. So, working with this project of course each of the projects have deliverables during this outcome. But we have tried to make a proof of concept of the objectives. So, the first level of the proof of concept is actually to make first constructs especially for liver toxicity as opposed to that was mostly experimented and predicted in the different projects. Than the integrated systems that are really predicting toxicity, they were a system looking at themselves, but it is best to take the toxicity human, then we have the application which is what we're talking about today and that is how you predict safety assessment from the different methods in these could be regulatory accepted.

The conceptual framework for the safety assessment, that is based on an exposure context. You look for the existing data and evidence, make a new process, you try to understand if it is general toxicity you're looking at. You try to understand the relevance of those internally, so you need to have some sort of predictions. Then you're looking at the toxic dynamics. Then you try to make an overall assessment including a certainty. In order to understand which guesses we have and such conceptual frameworks. You can make a safety assessment and hopefully apply that in an analytical context.

We have three different case studies for safety assessment. One is the threshold of toxicological concern and also predicting them. We are trying in the project and looks to have predictions and providing tools for predicting those. We have the read-across case studies which I will talk more about very soon and we have the case study where we don't have anything to read-across from. We are looking on the at the molecule and building out to make a safety assessment. That is of course the most challenging case. We met with a group of experts and because they are experts not only from that project but also other experts that have different aspects of with across, it was their first time in April and we're very happy to have colleagues also from the US with us in the outcome of this first meeting is published in HD and you can see the reference here on the slide. The group of experts come to the conclusion that we would need for typical scenarios that we would like to look closer to. They would more or less be able to apply read-across.

This first one, we have chemical similarities in the trying to compound but we have no metabolic transportation -- transformation so we assume that it is the -- itself. Then we have the chemical similarity involving metabolic transformation so that you have similar chemicals that are in some way transformed or are very similar. Then you have the third scenario which is no toxicity and you have the fourth scenario for you have structurally similar chemicals but based on the motive action you can distinguish these chemicals and probably this was different toxicities depending on smaller differences. You need a little bit more reasoning of how to subcategories your category anyway.

So, then the first step was to set up some sort of with across strategies so all the different categories of chemicals in different scenarios were described in similar ways so that is published in the paper that you see on the slide. First, to describe the rational of the target chemical in a transparent manner so you have sort of a template in the index of this paper, so you try to structure the information anyway that you can compare

different case studies. Then you document the logic and the data, and it can subsequently be re-created of course. Then describe the uncertainties in the prediction and you have to separate between the uncertainties related to the data because of course you will have uncertainties related to the data and then the answer contains the similarity and the uncertainties. And then to clarify the rules of any specific or endpoint for specific factors, this is sort of looking at the same endpoint of different endpoints that may affect each other, that maybe some sort of read-across the different endpoints or they can assist to have more confidence in the read-across.

I will give two examples for the different case than we have looked at, this is from scenario number one and the question here is if it is possible to read-across the 90 day study to the other compounds in this category, you can see that it is the chain length that is different in between the compounds and you can see that there is some with lower values in between those. Within the in between the data to confirm they are similar in that we can read-across to the target compounds in these categories from the source compounds. You have ToxCast data to look at these compounds in the different face. The nuclear receptor activation and the enzyme induction. That require similar at least some of the different chain links. You can see we probably have activation in all of them. Based on these data we see more comfortable that the mode of action of the compounds in these categories are similar and possible to read-across.

There is one thing that we're a little more uncertain about because we’re looking at rat toxicity. There is something that is quite particular with these substances. They have very different excretion rate in the different species. For example, in humans, and blood this can be elevated for weeks. And read we talk about hours. The question here is how they are related to each other and we would not need some more toxicodynamics evidence before read-across or the read-across not alter the chemicals in the category. But the nearest chain length and take away the chain-link that are the longest once. We have shown that you can make it across and you have more confidence in it. Please also found a gas would like to have more information. This is another example of the beta unsaturated alcohols. This is in the group of compounds that are metabolizing. They are going through some sort of alcohol do that dehydrogenase transformation. They are known to cause liver fibrosis. We look at them and we have a big group of unsaturated alcohols to start. We collected talks logical data and have these different computational profiles. One of the most interesting data we looked at is on the next slide.

This was a new *in vitro* model that was set up within the project. But it's aiming on protection of liver fibrosis. This is a coculture system in three dimensions where you have liver cells. When these are exposed during the 21-day experiment, you can see that the fibrotic substance that tend to start the show fibrosis in these little cell cultures. You can see from the one on the left, the control one that is the same. You have two different cell types and upon repeated exposure of a liver toxic substance what they look like. When we look at the system of exposure to six of these beta unsaturated alcohols, you can see clearly activity and they have these collagen formations. The hepatic cells are activated see can see the toxicity trend in these compounds and it's a

clear positive response in four of them. And two are showing less response. But those don't have the double bindings that might be the reason. We're going to investigate it further. This is an example or have the substituting an animal test for the prediction for the read-across.

This is the group of experts. We have other chemical categories were looking at that I will not discuss here. And perhaps one of the most interesting scenarios is the third scenario with low or no toxicity. And we have to groups of chemicals were looking at the primary alcohol and propylene glycol ether. This group is adjusted because these are the sorts of chemicals that we would like to be able to read-across because we have many chemicals like this that you would not you want to be sure that they don't have any toxic effects. Would like to have some sort of Safeway to say that this is good enough. With this data can do is improve confidence in nutritional read-across and suggest that smaller subcategories or more refined selection of analogs may be appropriate. You can better frame the category. They suggest what the initial studies might be needed in your showing the examples. So, you can strengthen read-across and reduce uncertainty. And discourage reliance on chemical structure similarity based only which can be quite misleading in certain cases.

I will point on some future experiments. We are soon going to be at the end of the first project. We have a symposium in Brussels on December 4. We will discuss the outcome and have a small Expo we can talk to the different contributors to the project. And you can register on the homepage. There's also been a publication about the recommendation for the future based on the lessons learned. That will be taken up for the horizon 2020 project the you talk risk 21. Then we illustrate the read-across assessment framework guidance that you can find on the ECH a homepage. They are now planning a topical scientific workshop for April next year on new approach methodologies and regulatory science. They will be looking at the application of the framework guidance and to best understand the underlying biology that how chemicals cause the fact and how we can show this, and it find new tools and techniques to provide the best assessment for regulatory applications. If you want to go to that workshop you can also look at the homepage and. With that I thank you very much and I'm happy for any questions. I hope to stay for the final discussion.

Audience Question: In the subject let me ask a question on the transition to the next horizon 2020 project. There will be much greater emphasis on developing and designing approaches that are *in vitro* and in silica to fit the predatory needs. Do you see the buy-in from the academics in Europe on trying to solve regulatory problems? Or do think it remains a challenge but they want to push the science and not the whether the science is advancing the regulatory agenda as well?

Berggren: It's difficult. But I think that there have been interest from academia to find that I that can be applied to regulatory context. Of course, this is something that it's a big project because then you will have people again in these horizons 2020 project representing many agencies. So, it stimulates the universities to focus in this area of research.

Use of Computational and *in vitro* Data in Cancer Hazard Assessment of Data Rich Chemicals: Examples of IARC Monographs
Ivan Rusyn, Texas A&M University, College Station, TX

Goering: I would like to introduce our next speaker Ivan Rusyn he is a professor at the Texas A&M University Department of veterinary integrative biosciences. He continues a productive research program there and training of our future toxicologists. He began his career at the University of North Carolina or he provided leadership to the curriculum in toxicology and was a director of the UNC Superfund research program. He has interest in mechanistic toxicology and as you can see by a number of these colloquia that he has planned, interesting computational toxicology and risk assessment approaches. He has served on numerous national and international committees and working groups some of which are the national research Council committee on toxicology that looks at 21st-century science. Emerging science for environmental health decisions. He has served on numerous working groups for the IR program on cancer monographs for different chemicals. He serves on the board of councils advisory group for NIEHS. Ivan, welcome. We look forward to your presentation.

Thank you, Peter. What I will try to do and bring this back to the actual data rich chemical. As we were planning this workshop we wanted to look into animal test replacement and to read-across when you have no information but not guess it there are many chemicals that are data rich. And so, this presentation will use the example of international agency for research and cancer monographs which is the largest and data richest of the packages that scientists are looking at. I declare no relevant conflicts. This is not reflecting any other organizations but my personal opinions. And the most important one these monographs are not published yet so part of the discussion in this monograph the presentation I will give today, and the actual language may change subject to additional editing.

Monographs are large effort and for those of you who know maybe that a few presentations I want to thank the Secretariat and the working group who commit to about one year of their life and preparing the documents and finalizing these documents. They have a finite time limit so at the end of that last day the smoke has to come out of the chimney and the decision has to be made now in 20 years -- you have to make a decision. I especially want to thank a few folks working with me more closely on these data. A code from Texas A&M, one from NC state and then others from the EPA. I have to thank the folks from talks 21 and ToxCast for making their available. The title says data rich chemicals. It does not get any better than IARC monographs. There is many information that goes in and it starts with all pertinent, all is the keyword. With any particular chemical or substance or lifestyle factor. If those animal study on guideline or non-guideline, it has to be included. This is the information that is available for a few compounds, but this is information that is something is the basis of the most decision. If you separate fact from opinion, you have the assessment to do that. And not just mechanistic data are not important of course that this is data are. We touched upon this. We fight on that type of information. Toxic appendix is crucial but as you start thinking about bringing silica data in figure about what it same. When I pilot here are the

parts of it was make an opening for this type of information. First is very recently IARC monographs program is started clearing mechanistic data with what happened the experts are invited who know something about it or something found in literature a systematic review. You have to look at the data that is relevant for comparing across compounds. Number how data rich or compound is the maybe instances where similar compounds in the free across similarities comes in. It might be better studied art types of assays. It makes you think of when it is relevant, or it wasn't bringing that information in. And then as far as where this information comes from, humans and animals and *in vivo* frequently. Some come in from *in vitro*. And is again is where the mechanistic domain overlaps. It's a review article but the comments are separate in brackets. These must be publicly available.

And here comes the database. What do I do with the database? There published papers not the methods are and the analysis but in reality, the rest of the information from thousands of chemicals and hundreds of assays is in the database. The key point here is that this has to be accessible. As long as it is accessible it's okay for the working group monographs. The monographs are data rich because what they're try to do is distill thousands of papers and tens of thousands of pages into a monograph that usually a few hundred pages long. The way to do so let down is to put it is you go through the cancer in humans and animals and mechanistic and other relevant data and this is the critical review part but the evaluation the summary and actual classification those with the working group makes a decision, but the decision is based on what is a monograph. You have to explain how you made the decision. You also have seen this before. I wanted to touch briefly that there are specific rules in the IARC monograph preamble on what sufficient and limited and adequate meet with respect to human and animal studies. We look at the mechanistic another data it's a different flavor of how a decision is made. There are two basic questions. Into categories evidence into weak or strong and second decide whether it's operated in humans. We will come next a why this is important. As a said before at the end of the day you need to place the compound in the chemical or into one of these categories. So, for the integration process are important.

The first cut of the classification is based on human data. If the data are sufficient in humans for cancer, then it is group 1. You don't need any other information, but you still would like to know what other information is out there. But frequently the studies are complex. Frequently there are challenges with interpreting toxic logical studies. This is what animal studies are done one chemical at the time for two years. This is easier to classify sufficient and limited inadequate. What you see here with the Aeros going up and down is mechanistic data cannot change the classification on human in an all-day or can up or down pretty evidence. But the important points are if it is strong information. Can operate in humans? Do we have evidence of exposed humans? And that's something that can elevate the chemical of way to class one because we reason we don't need to wait for 10 at the to be logical studies to make a decision on the chemical is without the precursor event was something that is linked to carcinogenesis and we have strong evidence that it happens in people who are physically exposed to a particular chemical. The strong moderate or week can operate in humans and there is

evidence it opens up the possibility to our thinking about other types. As primary information and as additional confidence in you not missing something for these publications that are crucial publications and not contradicting larger more comprehensive *in vitro* screenings.

The questions that come of these evaluations are, is it strong evidence? If it's moderate or weak it's nice to know. Or where does it come from? Is it from exposed humans? Are we talking about it in some sort of test that has confidence? We talk about *in vitro* systems humans and animal or some other *in vitro* system and all of a sudden, the screen test and they can provide some information that is useful. The mechanism only operated animals? I will show you an example of when Mr. type of information can be important for you to think through this process. Is will may only have some sort of model organism information but maybe this is something that we also have human cell lines and screening and I can show us theoretically if this mechanism is can operate in humans as well. And told read-across this is chemical similar to something already been classified? To be no editing logical studies because the exposure is so complex or groups of people that are exposed are small. So, on a case study the epidemiologic study world. All these questions are important. Additional ones also come up. Are their data gaps? When you go to the IARC monographs through the literature you found, but you need see how mechanisms have a lot of attention other mechanisms have very little attention. Somewhere just going about recently, we didn't have the tools study that but it's not just this is a mechanism that is not important. You have to think about are the caps because this is not the mechanism that is important or are the caps because there is the most attention paid to that particular mechanism? You can start thinking the screening is looking a much broader and more systematic than just one paper at a time.

Let's give an example of an early just 1.5 years old. The PFOA example. What if the mechanisms of the subgroup grappled with was there was not enough of review papers there is an opinion in the toxicology that activation is the only mechanism that is not relevant to humans. You have to as a member of the mechanistic subculture with these you might want to think about the human relevance of these animal data because you have done an animal study. One of the answers that the worker decided to pursue, and this was much a talk at the meeting for we were thinking about what type of information is there for us to answer this question. How to answer the comparative analysis of *in vitro* screening. We do that database and pull them information. These are couple of examples. We got the prototypical activators*receptor and others and the we looked at the relative potency of two forms that were screens. The morning assault and the active screening. We pulled those out and as you can see there in the maroon and red color on the top of each graph. What we're comparing them against is different types of assays. There's no such thing as one assay fits all. You think of the collection of assays. You consider potency is relatively similar, but they are the same assay every time. Mysteries at the start you would to other types of receptors of what you can see on the right is the oxygen receptor assay estrogen receptor assay. Other chemicals are positive as well. You can think about potency, but you cannot say that these are it's the only pathway that's being activated. We do the systematic review. You can conclude, and this is again I'm not sure the final language is because this is from a draft of the

monograph. The working group concluded that the analysis of human *in vitro* data is consistent with multiple molecular pathways being operative. With a particular question of the data rich environment but we really needed this type of holistic view of the receptor activation and these chemicals side-by-side in the same concentration range for us to actually answer that particular question in this particular way.

I mentioned that the IARC working group are doing a systematic review of mechanistic evidence in the 10 key characteristics of known human carcinogens. It's coming out in EHP. Is that unless I get but the prepublication cut email me and I will send you the draft. It also includes the instructions for authors on the bottom right. You can click on it and get this information. This is the information the reviewers and participant in the monograph would like to see. IARC 13 process of reevaluating all known human carcinogens which is known as volume 100 which is six books. Six different working groups want to all of the information of known human carcinogens and look at tissues that were targets of humans and animals. There were several meetings of another group of experts that look at all of these mechanisms and try to trim down into a number of events that we as scientists have confidence with the chemical treatment hits that particular path that we have a reasonable suspicion that there may be a cancer happening. This is not to be confused with the homework, so cancer are features of the cancer itself. This is more to be thought of as the etiology of cancer. I get to the features of the cancer cell. Again, this paper explains in greater detail and chose examples of how the thinking can be applied to go chemicals. This is likely glyphosate a lot of interest in this in this monograph was released in July. I should do this literature tree of the systematic review of the information that went into the chapter number four. There were close to 800 papers we identified as relevant and some of these are included in the monograph and many are excluded. But they were excluded for a reason. What you can see on the right are the 10 key characteristics. They are shown here. The numbers in the circles reflect the number of papers that of identified as relevant. For one of those people at this experience of models and human data.

When you look at this you say it of these 10 key characteristics only to a large number of publications. Maybe there are twentysomething papers on other things. But the rest of the key characteristics are zero. This is the moment, or you pause and start thinking is this a data gap? Are these characteristics not important? Where can I get additional information to increase my confidence I'm not missing something? Either positive or negative. The monograph working group exercise to upgrade or downgrade the decision made. In volume 112 more recent history March of this year, myself and other members of the working group we started thinking of how we could use the information on the *in vitro* assays and the overall workflow was to map these assays into IARC 10 characteristics. There's more than 1000 chemicals and 2074 assays. And then you have to think because this was all concentration response. We pondered for a long time when want to talk about potency or active versus inactive. The consensus was that the active versus inactive was the best were thing we can do. The next question is how do delete this information? One of the ways we communicate this we collaborated with them and published the graphical user interface, and this is an approach that has been described in recent papers one on the use of the trust is to ask those replace up to talk to the

studies. This gives you a rank order and a signal are there for your chemical in relation to other chemicals?

Let me take a few steps to explain this. This mapping of pathways you start with 821 and points. Many chemicals. And data are exportable to this by our standards is accessible. We dance them to 10 key characteristics. Were additional experts looked over our consensus and we came up with a consensus cross-reference between key characteristics and the *in vitro* assays. This table shows you how many assays mapped in our opinion to each key characteristic. The character six on the top and the number of assays is at the bottom. You start with 121 and points and only a quarter of them or less are mapping to key characteristics which gives you pause. We had those other assays and their important for other things. There are other endpoints. We start looking at the coverage for some of these key characteristics you have dozens of assays and for others you have very few and for some of these key characteristics like oxidative stress, we look at the assays can I trust this is what it does?

You also immediately see there are couple of key characteristics that are not mappable to *in vitro* or solvable with *in vitro* or in select models. You have to start thinking how we can do better in these instances. This was proposed by colleagues at EPA a number of years ago as a way to drive a signature of each molecule in relation to other signatures. And integrate across the assays and different chemical properties. At the end of the day you can then compare in the same set of information it's explained the bottom right graph or does the information come from and how me actual assays are in each slot? It 12 PM there's the TR which has five assays. Then when I have two chemicals you can see there's more action than with another combination. You can think of converting *in vitro* information to this in several different ways. If you have a particular assay and three substances. What you have usually is a concentration response. The compound on the left it every concentration with activation signal. If record as you go to the right, you have different chemicals. You can translate this potency into the size of the slice of pie. I bring all of the chemical on the left was called zero because the highest concentration was not toxic. And the chemical on the right was the most toxic or the one for which the concentration response was shifted most to the left. I'm going to sign that value one. Everything else is in between. I don't all of the assays to include and get the picture sickened that about relative potency but also three different groups or categories of compounds that are evaluated against the same assay quote receptors or end points and not only have similar potency, but they also have similar shape you can start thinking these are more similar to each other than across these different categories. And you have the ranking were if your compound is clustering with compounds of known hazards or the high-end the new need to worry about this, but this is a richly proposed as an observation tool. Let's show you a few examples of how we used ToxPi in this type of data to go through these characteristics among agents in the monograph evaluation. Here is the first conundrum to solve. What is the reference? I have five agents and I would like to compare them to each other. But you need to say they are probably not put together in this monograph because they are similar we did compare them against something else. So, compare them to other pesticides or two other IRQ line with chemical. The comparison is to all 1000+ chemicals and I'm showing

you three different pesticides. And without knowing much about what these particular slides represent you can immediately say there is more similarity on the chemical structure on the two on the left in the middle then enter by logical activity because the shapes and sizes of the ToxPi looks similar compared to the compound on the right. When it's put together from that thing it to the characteristics of slides that are of particular type.

Here is another view of the compounds but a good in them by mode of action. These all induce oxidative stress on the 11 PM and 10 him but if it other pathways as well. The activate other receptors. At the bottom are the estrogen receptor activation. And you can again start seeing similarities in the actual potency and activation of the same target when you view this this way. You have this information, so you should start using it. And again, this is downloadable and exportable. You really have to define the questions you are trying to address. What we were trying to do was match assays with particular character six to provided shall inside. We were not trying to make decisions based on this data on. User data rich chemicals which are to field data gaps and provide confidence in the information. That is important to point out. And finally, we are comparing them to the results of a larger compendium to get some bearing on where these compounds fault against other compounds with other similarities. In the actual volume 112 the compounds that had *in vitro* data with the pesticides I will show on the next slide. In this picture I'm showing you the eight inducers of oxidative stress. These are compounds that people don't question their oxidant stress potential. It was compared against all ToxCast compounds. We looked where the fall in these metabolites, they are not going to disregard. But that's not to say they are without information. They can do the same with receptor activation. We looked all of these receptors in the ToxCast screening. Within our compounds again some of them are without signal. You not only start thinking whether we're filling and data gaps but are the similarities between its metabolite and their compound. These are the top inducers and we start thinking about our chemicals under consideration there is only one showing potential signal.

This is a comparison against all chemicals in ToxCast but were in the IARC monograph world. We thought that this much of a relevant comparison is a gap chemical that have been evaluated by IARC and have been screened in ToxCast when you look at that number it's less than 200. Here I am showing you two different examples. And chemicals the top our top inducers and are shown at the bottom. You can also see where they are in terms of relative ranking. It's close to the top and then. On the right you can see the text with a work group concluded that yes diazinon demonstrated activity in both HR assays and estrogen receptor assays but the other only had limited activity and we don't know why this was. This is a data gap filling exercise and not something we were rushing to conclusions based on information. Volume 113 had a different take on the data, but they also used mapping of assays to key characteristics. They were dealing with another pesticide DDT and related. They realized that the right number of isomers and metabolites that have been part of screening. So, if you not only looking at the parent in the animal study or the humans of you can also start making comparisons across these different metabolites and isomers. Mechanistic studies may

have been done but in reality, they are similar. Can you read-across? What was concluded is that DDT and related compounds do share similar shape and overall rank and overall, they are much more potent select those is confidence in rely on traditional publications where you can use the information and read-across to other compounds from the same group. Finally, in volume 113 what you can see on the table this is the overall conclusion about traditional data. Originally it was strong and covered in humans can operate in humans. When we or that you can see the same trend. DDT and its isomer and metabolites are positive the large number of assays and that lindane and 240 are largely negative or not positive in the remaining assays. It gives extra confidence to the working group for the overall conclusions.

Let me conclude by pointing out something that is important. You cannot do this if the data is not publicly available. I want to stress and appreciate as much as I can't the fact that EPA and a larger talks 21 consortium are doing it think it's a job pushing this information out to the database and then through smaller download pages. All the way to the actual dashboard where you can view I want to point out to a number of sources the EPA dashboard demo was presented. The webcast was recorded so if you want details or if you missed on how to use this dashboard, I welcome you to go and go to the presentation on the hyperlink is here at the bottom. The final slide is to leave you with additional information on where the data are and how the analysis were conducted when the monograph comes out will be supplemented information with the actual cross-referencing and mapping and data. You're welcome to look at that. At the bottom are some examples as to how ToxPi has been used in different contexts. Thank you very much and I will take a few questions.

Roundtable Discussion
Moderator: Ivan Rusyn
All Speakers

Rusyn: As people are thinking about questions I want to invite our speakers for the roundtable discussion. In a quiet room after the long weekend. I would like you to start thinking about general questions. Please take a seat at the podium. While people are thinking about questions, those of you online please send them through the chat window to everyone and not to the organizer. Type it into everyone and then we will be able to see them.

Before we go to questions that were submitted I want to go around the panel and get a discussion on putting these tools into hands of the actual risk assessors and regulators or scientists. With that a presentation that were quite diverse in terms of there was a model and there's a database that is behind the curtain so to speak of the particular agencies. And then Richard yours was more of the these potentially can be used by others, but this is not something you can do yourself. And that the IARC example is a bit you can do this and start thinking or this information can be useful. Can we get a round discussion and thoughts about, are these tools ready to be given to regulators? Or do we need a lot of confidence building on the inside before we can run out into the general discussion? Suzy you are one of the organizers, so we can start with you.

Fitzpatrick: The key thing is for people to understand the tool. When you are busy with regulating or trying to get data outdoor meeting statutory requirement you don't always have time to look at the dashboard or think about. There're many things we could use this for. We were trying to use read-across with toxicology equivalents factors. Interest in that topic waned resources get to report something else and when it comes a big issue again. I do think that we are getting there. We have to start using some of these tools to fill in regulatory gaps and help us prioritize some of the resources.

Judson: We are working through three case studies with different regulatory groups to try to address, are we ready? The case studies are one was health Canada, so Ivan or Elizabeth talked about a meeting in European chemical agency in April. Can we bring together *in vitro* and *in vivo* and exposure data and so on to be able to do a rapid risk assessment? And then were doing similar things with pesticide programs at EPA looking at entered so active compounds there lots of animal data but the Internet compounds have relatively little. And they have been petitioned by outside groups to say something about potential hazard and exposure for these inert compounds. And finally, the Superfund group where they go into a Superfund site and can find hundreds of hundreds of chemicals may have to decide what is the chemical we will use as our cleanup target? Want to clean up until there is nothing hazardous at the level of cleaned up to so they need some prioritization tools. What we're doing is building databases that address those questions in building dashboards to let them easily get into the data. If you cannot get to the data, you might as well not have it. In principle as soon as we have those dashboards built and they are available in-house, we can flip a switch and they can be available to the external groups. I tell you now whether that switch will happen short-term or not. But what we're using this for is working with the regulators who are very busy, and they don't have time to tell us what they want. We show them something and they respond we iterate and so on and can do that rapidly.

Volarath: I agree with Susan. We certainly can use these tools. I think many people in the office now are open to the majority of these database tools. I think if we can bring the technology into where we are and show our team how to use them, and they can be comfortable and see how they can make their regular work application of these tools and I think they will start using it automatically. We just have to make the first connection.

Rusyn: Do see the uptake of this from the regulators or recommended communities? What it as about you're a Sprint about how SEURAT has translated to the actual application?

Berggren: I believe these methods can be used and can be accepted by regulators. If there using them in a lot of screening exercises. For example, we propose priority substance in the framework directive the most important pollutants to look for more information by using these competitions on methods. We have done a screening exercises to identify substances and also had a lot of use for the ToxCast database. In the classification it was the first that was used a lot but the incremental and points

because we did an exercise or environmental endpoints had to be classified at a certain point in time. So also, this is already classified for health effects. And you have to take the best you had, and you cannot request data for a substance. Then it was used and tested. And for pesticides you have data on active substance, but you have for the other components in the formulation and so on perhaps you don't. You need to rely on other methods. I also think we're going ahead. There is a large movement especially here in Europe. We've a lot of animal welfare organizations and we have these European citizens in the city when you have more than one might in people signing up for or asking for change in the legislation. And they ask for the third one that came through such a request. They asked for new safety assessments of chemicals. Of course, based on animal welfare but also based on issue that animal model may not be the best model for human safety. I think there's a lot of factors that is pushing regulators to accept the new methods. And it's very much on the research and scientific society to make the translation in a way that you can understand if you structure it in a way that the regulators can understand. Both what we're doing and why we believe it would be safe enough. Thank you.

Rusyn: Thank you for bringing something coming through the chat. Where can we help those, who collect the information and pushing this in helping the regulators that Susan pointed out don't have time to play around. There're decisions that to be made and you don't have all of the world is moving very rapidly. From the point of view of how your particular experiences have been in the question was directed to train 10. Is a better education or better interfaces or better data? Where is the shortest success story?

Volarath: I think the easiest way is can start looking at the data and incorporating it into what we do in the office now. We have to database. Once we can bring it additional *in vitro* data into the office and start show people how it can be applied to what they're doing now, then they can make the measurements or think to incorporate the new knowledge into their work. Another thing is an informatics team. One of the responsibilities can take on the state engaged with this type of activity and continue bring you the knowledge into the group and keep the group informed of the current technology and keep everyone.

Rusyn: Richard would you like to add on your experience with pre-dashboard and post dashboard communication?

Judson: I guarantee that busy people hate big spreadsheets and they don't want to even hear about relational databases. Going to dashboards is important. One thing we've done over the last year driven by our communications people is a lot of going out and getting user feedback. We set up several meetings with people industry, and other agencies, in US, in Europe, who would take the dashboard and play with it and critique it and it's gone easier to use.

Fitzpatrick: I think for example we classify things for a long time, but this is the first time I see how you use them. I did not realize the pie change with different endpoints. Now I see it's useful that I would not have taken the time to figure it out but these types of

seminars where we present the data and how we used, and this is useful. For people that don't take the time or those I was looking at the dashboard try to find information and I could member how I found it before. This is useful and helpful.

Audience Question: Something that had struck me was analogy to a powerful software package or musical effects processor. Many times, people are confronted with a lot of raw capability and what they really need to get started or to make it accessible is some model templates or model workflows. Even the case studies that were mentioned. Something like that that takes all the raw data or capability and put into a format where it's easily accessible and you can start tinkering with it and adapting it to your own use cases. But you are working from something that is relevant to a particular set of interest and drawn from the overall capability. I think the thing you mentioned about looking to and users to see what sort of things resonate with them or relevant and accessible. That's one of the biggest things that could be done to get people to engage more with this kind of data is to have more templates and model workflows or case studies around the bill can immediately see and start tinkering with. A point of departure if you well.

Judson: That is something I've learned from our communications people is an important point and what we have done is by listening to people and how they use this with put some online tutorials that are case study based. Ivy particular chemical and to know everything about it or I have a particular molecular pathway that I'm interested in. How do I start from there? It helps you learn how to walk through.

Rusyn: I think some of these EPA community of practice webinars especially the one I mentioned on the use of dashboards again is the report the particular use of a dashboard for a particular question. Definitely use cases and recording them for others to then follow is a very important of communication strategy. Another question I wanted to bring up is it was mentioned in some of our presentations that the level of confidence that they need. Asked the regular what kind those what level of confidence they want and there's no way short of 25 clinical trials to give that level of confidence. At the same time, I think some of the prediction accuracy that you are showing of 98% are pretty good. With a few questions on the web. Is there a gray zone as to when people start paying attention to a production of a model in terms of its accuracy versus it's nice to know but I'm not going to spend much time thinking how this helps me? Is there a 70% to 80% or 90% accuracy that you are targeting or is it we just need to communicate better with the accuracy means?

Volarath: I think the higher the accuracy is better, but I think it's one thing to explain the regulators because is the example of what were the criteria of what was the model and be clear. What the makeup their own decision and the transparent with everything that went into it and accuracy and everything.

Judson: One of the features of our case studies were walking through is trying to understand the uncertainty issue. There is quantitative uncertainty and if you have statisticians in the room. There are certain cases where you can throw really sophisticated statistical machinery at a problem. People do benchmark modeling and

you can say given this study, I think the benchmark is this possible bias a little bit. And you can have big arguments about that. What actually true is that there are other kinds of uncertainties that are more having to do that use rack instead of mice or a particular strain. That overwhelms the precision you get from that one thing. So, one of the things we're trying to do is be honest about all of the sources of uncertainty and many of which we don't entirely know how to handle what put warning flags out that there are reasons to not get too confident.

Berggren: I take the opportunity to boost. On this discussion I was thinking that it's important to contact the current uncertainties were accepting today and the regulators are accepting. In a way we're not looking for the full truth. Are looking for something that is good enough and perhaps we are currently using, or we have some uncertainty. For example, in assay like skin sensitivity for we have quite good alternative methods and strategies to put them together and at the same time we have a lot of human data. We can actually see that they are coming about the prediction of skin sensitization. We alternative strategies compared to the traditional test. I think there's a lack of evidence and a lot of work. I was thinking about how to speak and translate to an understandable language for regulators. I was also thinking there's a lot of cross sector interaction within these fields. You to talk in between different experts and that is also necessary to adapt the language and learn from each other. It stated more cross sector discussion between pharma, chemical, pesticides, bioscience to get the best understanding and to progress. I like the languages that is easy to understand so not intimidate the listener but actually put the text into something understandable by looking at ToxPi for example. That's a language is better to use when you speak to regulators especially. And then show the methods and amount of different papers and tell about all of your known what seems to be obvious to you.

Rusyn: I also think the whole level of confidence depends on what type of a decision you are trying to make. Argue trying to replace a particular test and then you may have a need for greater confidence. If you're trying to decide on whether there is a data gap, or can I get better confidence that might particular read-across or particular decision, I think the quantitative aspect of the information that might be gleaned from some of these *in vitro* methods is different. I don't think we need to be only thinking about the actual accuracy we think about which context were using the information and how much we can trust.

Fitzpatrick: And or the consequences of getting a wrong answer. If I am using it for a pivotal decision, then I want a lot of confidence. Is amusing for a screen or filling a get a gap or more information I live with more uncertainty. But really use the time at least as a regular type into the regular that question you're asking a screen might Tuesday go with one compound over another but a pivotal health decision. As you start using it lower level and that's how you gain confidence. I used for this was not a big decision. But it worked. I'm building my confidence up slowly where I use it for more important decisions.

Audience Question: Lori from Uniliver. Thank you to the speakers. This was practical, and I liked to hear all about the things. I like the 10 characters takes important in characterizing a carcinogen. Is there any plan on looking at establishing another set of 10-ish for noncancer as well?

Rusyn: I agree. I revealed a little bit. The original list was 26. The experts quickly realized that it's not a tractable number and people like 10 because of 10 thinkers or other comfort zones. But in reality, I think you point out a critical need which is if we cannot define the space in which we operate for a particular endpoint, we cannot really take full advantage of the other tools that could provide information to us. But given that define the types of hazards we should be worried about is also difficult. One of the attempts to systematize the types of hazards was undertaken in 2014 NRC report on the framework for chemical alternatives where the committee went through all of the different frameworks and try to make a finite list of the types of hazards for which you should be assessing if you're thinking about an alternative. These exercises are extremely important and of course the IARC cancer example is in some ways easier because there is a database of 40 years of known human carcinogens and detailed descriptions of their mechanisms of action in these monographs. And going through noncancer hazard assessments or other types of risk assessments is less of a certain exercise. But that doesn't mean we should try. Should not try.

Judson: I wish. The endocrine program is simple in that there is a for the pathways you have to look at. But if you look at general toxicity we can define an adverse out those impact outcomes. No one has a clue what the endpoint is. If you start looking at *in vitro* data for *in vivo* data and the complexity that, maybe we will get to your 10 noncancer. I don't see have to get there.

Audience Question: A second question. This one might not have as much of an answer. You all have identified that low toxicity or no toxicity compounds were problematic especially in the European example. Of how to go about framing *in vitro* and asserting safety or read-across even. Because there isn't much toxicity to capture and read-across. Do you know what type of plan there is to address that? On coming from the food side as many of you out there are. Our whole thing with food is that it’s non-toxic. Had would prove its negative?

Rusyn: Elisabet would you start on this answer? This is quite a discussion that we had last week at the JRC workshop.

Berggren: Yes, thank you. It's difficult issue. Effortless to what happened so far in the discussions, something is used on the market for a long time and so on. So, have a certain confit level that we don't need so much data. If it's something new they need a lot reasonable data to show is negative. Or very low toxic. And you can come into these equation and just produce more and more data and it seems like it's difficult to satisfy all concerned that I wish to see some sort of in the future, a package of *in vitro* and in so ago assays together that tested on a large set of substances with is that NEC these filter is good enough to say that if it's not showing something particular. We would see

those we would have the comfort level to say that this is low toxic enough to release. But I think there's a lot of work there's a standard package that could be used but that's really useful to try to work on.

Rusyn: I agree. The confidence comes from a package of information that is holistic or comprehensive. And comprehensive may mean model systems that cover different pathways or different organs. And also looking broader than just one particular readout. From the high throughput genomics capacity comes online, I think that holds a lot of promise. On the flipside I think there is a pretty from school of thought in read-across that you are reading across for one particular endpoint and you're not supposed to look at anything around that. You should stay under that particular light post. Where I think I'm of the opinion that to really prove or to increase confidence you have low or no toxicity, you need to take the compendium or comprehensive approach. To demonstrate that we've visited and a large enough said that we've had quite number of positive compounds cover different modes of action and then we see memo and all these assays. I don't think the coffins will come from one particular test or model. It's the challenge as Elisabet pointed out and there's some potential solutions but it won't be an easy as I disprove one particular toxicity.

Berggren: May I add something on that. At the end I think is a lot of these things are coming from biologically available and what can be biologically active. Even if you would look at the different endpoints and so on, at the end when it's been tested enough, I think a base set of tests could be quite small. One of the models that was proven to be useful and accept it is the different barriers in the body, so you understand why it can reach and of course the first one is skin.

Judson: On the issue of can we prove safety. Much of what we do is prove toxicity. Many cases you would rather prove safety. The approach we are proposing, and we will try this out is high throughput genomics. Within the next few years it will possible to do whole genome transcript elements for a few dollars personable. We will start being able to run thousands of chemicals with many different cell types including metabolically competent cell types and primary cells and cell lines and so on. And the concentration response. We will be able to say nothing happens in this chemical. As far as we can tell, and we put hard. There is no exposure scenario we can find or reasonable exposure scenario. Therefore, under the conventional way we have used the word safety, that chemical is safe. The reason for being willing to say the save word which we were not ever a lot to say that word. Maybe FDA you're not out to if you don't to that you're left with people on the entire mental side that all chemicals are back. We're all going to die. But if you really believe that you would never have the resources to focus on the truly bad suspect chemicals. We have to find me say under certain circumstances, under one of these areas this chemical really is safe and the conventionally understood way. Having said that there is a study that is getting ready to be submitted that the hindrance is copy fruit and veggie study. They are safe. We them all the time. They took a bunch of broccoli and carrots and potatoes and blueberries and make sure they were organic and washed them well and the ground them up and put them in look and they are really bioactive. You can understand because pesticides or fruits and vegetables evolved so

they are full of natural pesticides and bioactive compounds. And we them because they are antioxidants and so on. The study is interesting. What the safety really me? I don't know the answer to that.

Audience Question: I want reference that last study which is the important one. If we're going to be looking at you assays, we need to understand their domain of applicability which includes how things we think we know about reactive and not just toxic things. Everyone wants to test as a positive role the blazing positive and expect the control salt and sucrose. I think we have to have a wider understanding of what a negative control is ever trying to understand on USA. Reacting to Dr. Bergman's talk on thinking about the chemicals that are most data rich which the pharmaceuticals are. What we have learned from our cousins in Pharma, they do computational toxicology all the time. They generate libraries of tens of hundreds of thousands of chemicals. Only a few hundred of which work even though based on competition of toxicology they all showed. Before we get too smug about putting down competition toxicology, we also have to recognize that this of chronic and chronic bioassays we use the throughput drug candidates in patients are terrible operating target toxicity. Most clinical trial scale because of that. And to the relevant assays we talk about are not gold standard they are just because we want to replace them is not replacing the great test. As we talk about new technology that comes in, the question is not does it give the same results as the other as a because you're holding it to rock standard. In the IARC monographs the need for two weeks and come to a conclusion. The nice thing about the IARC monograph is yes you have the page of which they also have 300 pages of data on how you came to that conclusion. I think that's what's important about the IARC monograph is that you can go back into the data and asked do I agree with their judgment or not? And so, as we try to validate new models for use in risk assessment, that degree of transparency amongst the regulators. The problem with the new technique is that you have a relatively small group of people that develop it in they are not objective about that technique. You have to expand the group of people who view technique and then have some transparency about the process of evaluation and acceptance. That is the key to acceptance of new technology into risk assessment. Are convincing people that the group to review the data was a disinterested group that was not went to this particular method that they are reviewing. And having transparency to the review and how they came to their conclusions.

Rusyn: Thank you for the support method for point and I think that certainly would go through a lot of criticism in the questions of transparency that I think in every case have been resolved where the information is public available and even if people are using it and different method of analysis, then ultimately if you compare apples to apples or oranges to oranges, you come up with the same answer. In the future tox meetings we had presentations from the other side and we analyze them in a big a slight different conclusion, but the results were largely the same. Richard do you want to comment on this? I think there's a lot of confidence now and no questions of lack of transparency because of the dashboard. But it has not been like this forever. Can you comment perhaps on this point?

Judson: We continue to get criticisms about lack of transparency. We find frustrating because we are providing more data than about anyone else we know. One of the reasons is we pay labs to run our assays. These are contract labs and they don't want to make all of their SOP's publicly available because that is their intellectual property. We need to respect that because they would be out of business if they cannot protect that IP. But we've been working with them and more and more of the SOP is available. After the and time someone got up a meeting like this and said no one should use our data because we cannot what upset transparent. I have to ask some people who get the guidelines studies. And I go to a CRO and get their detailed protocols? Of course not. All of the animal data we use all the time is run with SOP's that are not publicly available. There is nothing new here in trying to predict some of the intellectual property.

Rusyn: Patra, you want to weigh in on the oversight understanding of where things are coming from and how they are organized?

Volarath: Yes. That's a level of confidence. I think we can display some level of transparency to the outside field. I think to be as transparent as you can that helps. I know you can't reveal everything but the more we know the better.

Audience Question: Mike from house Michigan State University. I want to thank the speakers and organizing committee. I like this discussion. I think drop what I wanted to contribute in the way of the question. And when Ivan was talking. I'm impressed with the advances we've made in modeling computational toxicology. I think will have to be provided as start thinking about safety. Peristalsis the ancient father of toxicology said that the dose makes the poison. I'm worried we've invested so much in modeling the hazards that I would like to hear some impressions from the speakers and perhaps from the audience. Are we doing enough to really model the exposure side of the equation? If we don't, we're going to be facing regulations based on half and is on a good place to be. I would like to hear some of your impressions as to what we are doing with modeling exposure. Goes on adjust exposure in the environment but even some of the *in vitro* to *in vivo* extrapolations. What are doing about exposure modeling? What more could we do?

Judson: The folks I work with at the EPA have an equivalent program called Expo cast to move exposure initially it was let's do high throughput and do something. As we have done that we have got more and more sophisticated on low throughput exposure modeling. All of the modeling efforts have shown that you can be exposed in two ways. One there is something out of the air. There is a factory that is spewing something into the air or water. And there are things you bring them into Houston paper walls or put makeup on or brush her teeth whatever. Most of their exposure comes from those things you've potentially but and brought into your house. That's all the modeling efforts tell us. There certainly are if you within a community which is sitting next to a Superfund site or communities with exposure is more incremental. But for most people in the US and the developed world most of your exposure are coming from something you have intentionally bought. One of the uncertainties is that. We been doing a lot of hunting looking at materials safety data sheet. Every part that Walmart sells you can download

MSDS and find out all of the potentially hazardous chemicals of the fraction of the chemical is in the product you buy. So, when you put it all together and dump it all into a exposure model, becomes an bigger set. Then how much of this product is actually canceled? We can work with Walmart cut the have given UPA access to sales figures by ZIP Code for every part they sell. It's a one-year snapshot. We can actually say this product contains this chemical at this level and in this ZIP Code. People but a lot of it. And another neighborhood people but only a little. We're starting to see these enormous data sets that are out there that could potentially go mine and alert people there's something that is out there that is hazardous and there's exposure scenario we can see through this modeling effort that we should be worried about. That's one big approach.

Rusyn: I'm glad you asked this question. The new data *in vitro* and in so it does not stop faster problem. There are approaches to model exposure and try to better understand what types of scenarios people may encounter so you can Crutcher has a prediction from that. It's important on the other side to go and get the measurement. Even though we haven't talked about that part in this particular series of colloquia, the NRC committee on emerging science of mental decisions has a series of the Temple mix workshops. What happened earlier this year was for 1.5 days there was a bit of discussion on how to solve the actual measurement problem. I just environmental exposures but also the internal doses and concentrations. The discussion about targeted analysis from things we know of we start doing untargeted analysis to really understand what things were exposed to and what things are in our bloodstream or tissues. It's a difficult challenge but in some ways it's not any more difficult than the hazards challenge. I agree with you that there needs to be attention paid to the exposure side of the risk. For that there are other publicly available avenues of workshops. Of the NRC talks are recorded and put on YouTube. We need to be thinking about that and talking about that brings particular cochlea we focused more on the hazards part. Elisabet you have any comments for this question?

Berggren: We for a lot on these *in vivo* calculations. To try to combine into experiments to make sense of them. Of course, in that sense exposure is important related to the *in vitro* reality. And also, the route of exposure is important to understand how it enters into the body. Because of that which type of internal exposure you can expect. Then I think that something else that is more the level of how you make a safety assessment is of course exposures scenario you set up would probably take a different type of safety assessment. How much uncertainty you could accept a new assessment depends very much on the expected exposure. Of course, exposure is important to get right from the beginning.

Rusyn: Thank you. In the last few minutes if I could get some quick reaction from the panel on the topic we touched upon quite a bit which is the challenges and limitations. Not just on the uptake of this information but challenges of limitations on the actual production of the information. Throughout the toxin for the metabolic confidence of our *in vitro* models' systems. The coverage of the insular go model systems. Which are perhaps and CCT has been in the forefront and taken the front of the criticism we set that this presentation. Yes, I know. These are things were not covering. But a few

lectures start thinking about things you can and cannot do and could you perhaps comment on some things are not going to be solved in our lifetime with others.

Judson: Predicting something cannot be done is always a bad thing for scientists to do. Someone comes on and tells you how to do it. I will focus on a few things which are difficult. We had some ideas on how to build metabolically competent systems. But that has not happened yet. How do you take *in vitro* potency and turn it into an *in vivo* potency? To duty pharmacokinetics. We have lab and modeling work going on. The errors in that methodology are still quite high. And you don't know which chemical we can't trust the answer for in which we cannot. Some people think it's true but it's not there's more that needs to be done there. A practical problem which amazes many people is there might be 30,000 chemicals that are man-made chemicals to which we are exposed. It's not that huge. But we set out to buy. We start off with a list of 20,000 chemicals wanted to scream. Most of those you simply can't buy. You can't go to the vendors and by 1 g of something to her. You can buy a tank load or nothing. Getting access to chemical samples to test. We could test 30,000 chemicals but we cannot get access to samples. That's an issue. And approximately going back to the exposure issue, there are lots of the questions. In particular if we could of methods like Dean Jones at Emory can go and detect all the chemicals in our blood. The high-resolution mass spec methods but he cannot identify the mapping. That would revolutionize exposure science and toxicology. But that's a big open question.

Fitzpatrick: I agree with exposure is often overlooked. I think that's why exposure modeling it keys in on whether exposure or toxicity data were looking at or we need. It people are not aware what is then proceeded to look into it. Once we demonstrated it was a very effective tool. It's a mixture and looking at chemical by chemical. That's the biggest problem with food. It sits in soil and water and perfect is not where you know what you applied. How do build comfort? Food is it additives or contaminants? And were those contaminants washed out in the handler? With exposure in food is the biggest. You don't know what's there or how they interact. And maybe with some of these height speed data we can look chemical by chemical, but it doesn’t really give you the real answer.

Volarath: In terms of limitation we have the data coming out. We a lot of different types of data sets that we can look at. If there is a way that can cross-reference information between data sets, that can help out in expanding the exposure data. I think when we can cross reference on the data sets available. We can cross-reference at the chemical database available and see what kind of information that can be pulled from the cross-reference. That could be a key or one way of overcoming some of those limitations to the data.

Rusyn: I would like to thank the presenters and Elisabet thank you as well. Thank you for joining us and thank you for participating here in person and on the web. We have final words from our president.

Goering: Thanks Ivan. As President of the Society of Toxicology, I acknowledge that we heard some real nice presentations. We've heard some good examples of how we are making progress and how we're going to use these *in silico* and *in vitro* approaches to help us ultimately make better risk assessment decisions and better regulatory decisions. I want to thank the speakers for the time they took to present to us today. Thanks to Suzanne and Ivan for organizing this session and taking the leadership along with members of the organizing committee. Thanks to Betty Eidemiller and her staff today and headquarters workers who helped in the background in the weeks preceding to pull this off. I also if you're interested in more of the same of learning about the progress we're making in this area, I want to call your attention to another SOT sponsored meeting. Future Tox 3 is a local meeting in Arlington, Virginia. Registration are open now and we hope to see many of you there. I want to make you aware of the recordings and slides will ultimately be available for everyone to download. I also mentioned the survey. I know we're all surveyed to death but if you registered for this meeting by WebEx or in person, you will receive an opportunity to take part in a brief survey. It will be helpful if you and your ideas of what kind of sessions or programming will be helpful to you in the future as we continue this partnership with the Food and Drug Administration. I thanks to everyone involved and we will see you next time on December 3 for the next colloquium.