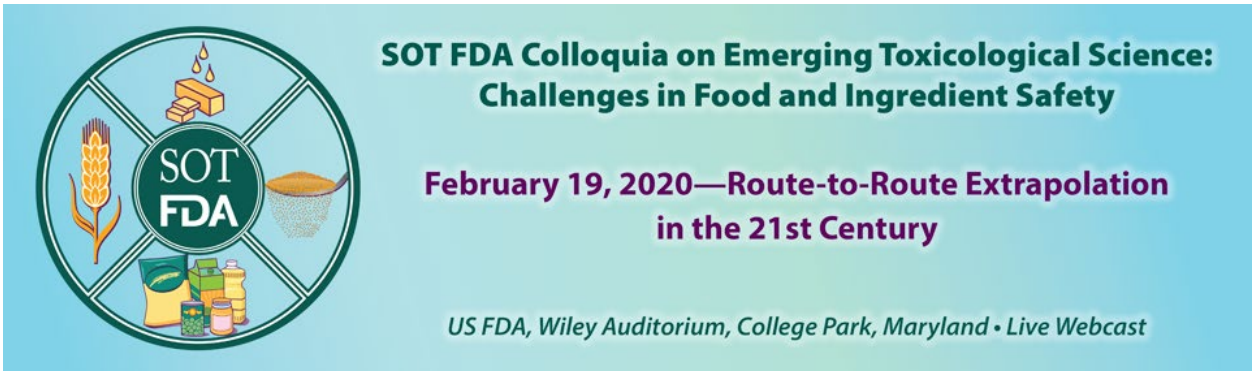

Real-Time Captioning

Note: This is not a transcript.

February 19, 2020

Route-to-Route Extrapolation in the 21st Century

8:30 AM–8:40 AM	**Welcome** Jason Aungst, US FDA, College Park, MD **Speaker Introductions** Harvey J. Clewell, III, Ramboll, Research Triangle Park, NC
8:40 AM–9:15 AM	**Introduction to Route-to-Route Exposure** Harvey J. Clewell, III, Ramboll, Research Triangle Park, NC
9:15 AM–9:50 AM	**OECD Guidance on the Characterization, Validation, and Reporting of Physiologically Based Kinetic (PBK) Models** Alicia Paini, European Commission's Joint Research Centre, Ispra, Italy (via webcast)
9:50 AM–10:25 AM	***In Vitro* to *In Vivo* Extrapolation of Metabolism Data to Support Physiologically Based Modeling for Route-to-Route Extrapolation** John C. Lipscomb, Center for Toxicology & Environmental Health, North Little Rock, AR
10:25 AM–10:40 AM	**Break**
10:40 AM–11:15 AM	**Determination of an Internal Margin of Exposure between Rodent Oral and Human Dermal Exposures to Phenoxyethanol Using**

	Physiologically Based Modeling John Troutman, Procter and Gamble, Cincinnati, OH
11:15 AM–11:50 AM	**Examples of Route-to-Route Extrapolation Conducted at the FDA Center for Food Safety and Nutrition** Shruti V. Kabadi, US FDA, College Park, MD
11:50 AM–12:50 PM	**Roundtable Discussion** Moderator: Harvey Clewell All speakers Jeffrey Fisher

Welcome
Jason Aungst, US FDA, College Park, MD

Thank you and good morning. Welcome to the SOT FDA Colloquia on Emerging Toxicological Science: Challenges in Food and Ingredient Safety. My name is Jason Aungst and I am one of the FDA liaisons on this SOT FDA joint venture. I will be monitoring the webcast for any online questions you may have. During the roundtable, feel free to direct any online questions to me in the web chat. This series which we've been holding for number of years is supported by a great partnership between FDA and SOT put our goal is to have a good scientific discussion today but were not going to be discussing any regulatory policy issues so try to remember to keep your questions a discussion focused on science and methods presented.

This colloquia is supported SOT mission is shown here. And all previous presentations are available online at the SOT website. This is a great resource to go back and see some of the other interesting topics we have covered. I also would like to thank the organizing committee for the work behind the scenes and especially Jieun Lee for raising the topic for discussion today. Now, I would like to turn it over to our chair, Dr. Harvey Clewell.

Speaker Introductions
Harvey J. Clewell, III, Ramboll, Research Triangle Park, NC

Thank you.

Good morning. I would like to welcome you to this colloquium as well. As you can see, we have a fine list of speakers that we are going to, I'm going to begin by giving a brief introduction to the area. Then Alicia Paini from the European Center for Validation of Alternative Methods will be talking about the current regulatory guidelines for use of these kinds of models, PBK models or PBPK models, everybody argues about the acronym that we should use but we all kind of agree on the value of it. Then John Lipscomb who has had a distinguished career in the EPA is now semi-retired in Little Rock, Arkansas, will talk about the key elements of using PBPK modeling in the future, *in vitro* to *in vivo* extrapolation to obviate the need for doing *in vivo* animal studies. Then, we will have a break. After that Shruti Kabadi, oh I guess, oh yes. John Troutman, sorry, will be giving an excellent example of the use of PBPK modeling for extrapolating between oral rodent exposures and human dermal exposures in support of a cosmetic risk assessment. And then finally Shruti Kabadi will providing

examples of route-to-route extrapolation performed in the FDA Center for Food Safety and Nutrition. At the end will get all the speakers back together. And we will have a roundtable discussion and my friend Jeff Fisher, Dr. Fisher is a senior scientist at the National Center for Toxicological Research in Arkansas, he will be assisting me, he is on-site in Arkansas today, but he and I will be moderating the discussion and you will have an opportunity to ask questions about the subjects that were discussed today. We will also have time for a few questions directed at individual speakers for clarification only. The discussion point should be saved for the end.

Introduction to Route-to-Route Exposure
Harvey J. Clewell, III, Ramboll, Research Triangle Park, NC

So, I will begin my presentation as I said the focus of this colloquium is on extrapolation. There are many ways that that is been done over the years. The needs of physiologically based modeling have been able to provide a much more concrete and biologically based subject for doing it. The whole goal of this kind of modulating regardless of whether it is a cross species or cross routes is to try to be able to relate toxicity on the basis of target tissues to actual site where the effect of the chemical occurs but this may be the chemical itself or one of its metabolized or the reactive metabolized forms for example in the liver. The point is that the relationship between an inhaled concentration and an ingested dose of dermal concentration on the skin is complexly related to the concentration at a particular target whether it is the long, the liver or the brain. PBPK modeling is the attempt to describe that relationship quantitatively. Which allows you to have a concentration at the target tissue that can be used within adverse outcome pathway to describe the dynamics that lead to disease. The eventual goal is to describe all of this quantitatively in a seamless fashion.

It is really a chemical engineer's view of a human. The model structure is based on anatomy, but it shows parallel blood flow to the tissues. It comes together in blood and then goes through the lungs. And then arterial blood. The metabolism and transport processes are modeled as necessary depending on what is controlled for this position of a particular chemical. A metabolic clearance usually occurs in the liver and is modeled there but it can also occur in other tissues where it may be important for the local effects. A biliary escalation might be necessary, it may be that it plays no role for a particular chemical. The model parameters are available in part from the literature. Physiological data has been selected over the years for organ weights and blood flows. The biochemical data has to be determined in other ways. Partitioning can be estimated using quantitative structure property relationship. Metabolism can be determined *in vitro* with either parasites or other preparation. So, that provides either the parameters, and the equations themselves are fairly easily put together on the basis of mass balance. How much chemical comes into the tissue, how much chemical goes out from the tissue? Under normal circumstances it has to be equal except for what is lost by metabolism or a clearance processor. The model actually is prepared from the diagram for an example diagram being on the right shows the long at the top, the liver and fat split out from the other rapidly and slowly perfused teachers rapidly and solely prefers refers to just the relationship of blood flow to the organ volume. Still the rapidly perfused tissues like the liver and the kidneys have a high blood flow and so stay in relative equilibrium with the blood. But we have to separate the liver because that's where metabolism occurs, and the blood flowed to the liver can limit the amount of metabolism. That has to be a separate compartment.

The tissue includes the muscle and the bone morrow. Again, fat would be in that compartment because it is certainly low blood flow compared to its volume but we have to have it separate in

many models for chemicals that are either to fulfill its which therefore would be accumulated in the fat or are lipophobic and therefore would not accumulate in the fat but not even going into the facts. So that would change the distribution volume. There is one equation for each tissue represented at the box in the diagram. There is interconnected by the equation for the blood. Just an example for those of you who are not afraid of equations, the example of the liver, the change in the amount in the liver with respect to time. That is VA LVT. Is equal to the blood flow minus the arterial concentration feeding the liver minus the concentration leaving the liver in the venous blood divided by a partition coefficient that represents the equilibrium serving the dynamics for that chemical it is partitioning.

Then the second term is an example of a metabolism equation that would remove a chemical from the liver by converting it to a metabolite. What is shown here is the equation where you have a maximum velocity times the concentration adjusted to the preconcentration with a partition coefficient and that is activated then by the affinity KM plus the same free concentration. That metabolism is often very important in affecting their relationship between those by a given route and the concentration at an internal tissue. In general, PBPK modeling is used to define a relationship between an external measure of administered dose or exposure and an internal measure of the biologically effective exposure or dose in human exposures of concern and in relevant toxicity studies. So, risk assessment is a ratio business in order to infer human risk from whether it is an epidemiological study or an animal study one needs to be able to predict the internal dose that is produced both in the toxicity study that identifies the dosage at which effects occur and in the human exposure of concern. The value of the physiological based model is that they can incorporate what we know about species and exposure and differences.

he advantages of PBPK modeling is blood consistent use in recent days. Internationally for risk assessment. Chemical risk assessment, drug development research and evaluation, interpretation of human biomonitoring data, *in vitro* to *in vivo* extra population of toxicity vertically in support of Tox21. The toxicology in the 21st century. And evaluation of early life susceptibility, EPA Office of Pesticides uses PBPK bottles *in vitro* to *in vivo* extrapolation as an alternative to animal studies for determining early life susceptibility. Of pesticides.

So, the most important impact as I said, is for use across species and cross routes of exposure. The challenge that is involved in using PBBK modeling is you have to know something about the mode of action of the chemical. It is important not to ignore the fact that some chemicals particularly drugs for example, have their effect due to the parent chemical exposure. It is also true of some non-pharmaceuticals like dioxins and nicotine where the effective chemical is what you are exposed to a metabolism is basically DC toxic vacation. That is one case. A quite common case particularly in the environmental world for cosmetics and personal care products it is actually a circulating metabolite that produces the toxicity. An example is the filing through the monophyletic's causes inhibition of Tet tester and production.

Trichloroethylene has been metabolized all of which have been bad for you. So, trichloroethylene excels causes neurological effects, causes liver effects. You have to know something about the chemical and actually do a good job of conducting a risk assessment. The relation across species and across routes will be critically dependent on whether it is the parent chemical or circulating metabolite or a reactive metabolite such as in the case of trichloroethylene. Vinyl chloride, which is highly mutagenic, chloroform which is metabolized which is highly toxic. It is a necessary element of doing good risk assessment to understand how the chemical causes the effect in the mode of action is determined developed by the EPA

to describe what duty to know about the chemical toxicity mechanism in order to be able to conduct a reasonable risk assessment. Nothing more, nothing less.

Moving on to relics population, there was an EPA sponsored meeting in the late 1980s that brought experts together to talk about how to conduct route to route extrapolation. Over the years it has been done fairly simply assuming 50% take by oral, 52 100% uptake by inhalation regardless of the chemical, regardless of the physical properties. So, there was a recognition that it was not being done well. So, this decision tree was developed at that meeting. As you can see, begins with the question is adequate doxology data available for at least one route. If not, then one would have to use structure activity relationships, near neighborhood comparisons across and if not, then you're probably going to have to collect some data. But if you do have data, then for one route and you are trying to extrapolate to another then the next question is the toxicity from contact site likely?

So, what this means is this a chemical that is likely to react in the long like ozone before it gets to the system circulation, is it something that would react in the gut, in the stomach before it actually gets to the circulation. If you're talking about a portal of entry effect, then you really cannot do route to route extrapolation. Then you need data. But, fortunate, there is a pathway on this that comes out with a yes. That is if the data suggests that the toxicity is remote to portal of entry. This includes the liver which is closely associated with or uptake but still it is an internal organ with internal system chemical blood flow. So, any of the traditional target tissues liver, lung, brain may be considered a remote tissue. It depends, again, on the mode of action of the chemical weather qualifies. So, this is something that requires some expertise and some information on how the chemical works. So, getting more particularly into the option where you can do route to route extrapolation. What has been done to racially was the default absorption values. As I mentioned, for example, 50% uptake of environmental chemicals is often assumed for oral exposures. For inhalation exposures, the common way that it used to be done was to take the concentration in the air, multiplied by the ventilation rate and then assume anywhere from 50 to hundred % of that is absorbed. And alternatively, there could be measures of absorption efficiency.

And use of an internal marker for bioavailability. They the recommended approach from the CPA sponsored meeting was development of a comprehensive delivery dose description read PBPK model. So, there is reasons why it is important to consider differences in the different routes. For example, inhalation and dermal uptake are directly into the systemic blood. The inhalation of course goes primarily into the tracheobronchial pulmonary region and then from there to the arterial blood. The dermal uptake goes into the veins returns from the skin and then into arterial blood. On the other hand, so it bypasses pre-systemic clearance which primarily occurs in the liver. Oral uptake goes through both the gut tissue and into the portal blood and then into the liver where it goes through the liver single pass before going into the blood the systemic blood but after it gets into the systemic blood only 25% of the chemical goes through the liver. So that pre-systemic clearance where 100% of the chemical goes to the liver can have a high impact.

For many chemicals metabolism and the gut is quite substantial in parasites. There are programs that can predict these effects. And a former they also use things like SimCity program. Like some sip which is a form illogical platform particularly design for drug interactions, but which comprise a good platform for any kind of modeling. There are important differences in the metabolism by different routes because both inhalation and dermal have very limited metabolism. It can be very important for portal of entry effects. The lung tumors from methylene chloride are due to the metabolism in the lungs of methylene chloride. Apart

from portal of entry effects they can also play a small and limited role in affecting systemic uptake. Whereas with oral, there can be very high levels of pre-systemic clearance.

Just to illustrate all of this I'm going to give an example of a safety assessment for acid. It was performed at the request of the FDA Center for Drug Evaluation and they were concerned because they had received a new a request for a new application of retinal a acid. For screwing cream. It had been used for years in treatment of a particular form of leukemia, but it also is just a different isomer of a chemical that had been used for acne. So, they tried using, the company wanted to use all trans retinal acid as a wrinkle cream. So, FDA obviously was concerned because of the relationship of actually the 13th in the alt-right summers into convert in the gut. They did not want to have teratogenicity; they wanted some assurance that the level would be of the chemical that would be achieved for skin treatment would not cause would not have a potential to cause teratogenicity. This was at the point where the FDA was in the FDA was unwilling to go forward with labeling until they had resolved this concern. The problem was that the only data for humans was radial label studies which were unable to discuss the grace that active metabolites. It was total all trans retinal acid plus any oxidative metabolites plus click here on nights. And they do not cross the placenta so it was not possible to determine what the effective dose might be.

So actually, Carl Peck was head at the time, and he recommended the use of PBPK modeling to provide him with the assurance that you needed. So, the approach then was to compare the fetal doses predicted by the model that would result from the maternal dermal use against those associated with teratogenic the city. This is a diagram of the model, it is much more complicated model than I showed you before but it is still the same concept there are various tissues, in this case at the top where the plasma, we have done two compartments for the skin that go into the bio epidermis where the blood flow joins the skin. Then there are the richly perfused tissues and then we have the placenta and the fetus. The reason there is more compartments is because we are trying to model the skin compartment and the fetus with this model.

Then when you get down to the liver, it gets complicated because the metabolism of retina to is complicated. There is also sidechain oxidation which I did not mention. So, we have to be able to describe all of this with the model. Fortunately, the people at the pharmaceutical company that had been working on this had conducted a huge number of studies to characterize the metabolism. They also had pulled the other available literature and so that greatly reduce the problem of identification. Again, the physiological parameters were available in the ledger bird there were oral mouse studies and ex vivo human placenta study. There were metabolism studies in both rat and using intravenous administration. In the and also in rat and their work oral studies where they identified the metabolite production. They actually measured how much metabolite was produced. There was data on biliary excretion from exterior eyes the bile duct studies. Urinary and fecal excretion in both rat and human studies and it was dermal exposure studies in the human. Then in the human oral and topical exposure parameters and human oral and topical studies *in vivo* and *in vitro* studies.

So, this shows some of the data that was used to identify the parameters in the model, the genetic parameters. The rat introvert fetus study and the intravenous. You can see this is stature metabolism as one would expect for all trans retinal acid. Sit metabolism oxidative metabolism in the rat and primarily Google ligation in the human. That turned out to be a very important difference. This shows the human oral data actually from a study of volunteers with leukemia patients. Then the dermal study showing the plasma concentration in the middle and the fecal and urinary concentrations on the right. So, what we found from all of the modeling

was that there was a very important speech is different in the predominant metabolism of all and in ruins it was oxidation to an active metabolized this was still an in primates it is a very high affinity glucuronidation to inactive form. There were also exposure differences in bioavailability, oral uptake was very rapid and could exceed the capacity of the high affinity cougar on dice pathway and so that would cause more of the chemical to be absorbed directly at the higher doses. Whereas slow dermal uptake was uniformly subject to the high affinity glucuronidation clearance.

There were important differences also between the all trans isomer and the 13 CIS Accutane which help to explain the difference in toxicity that suggests there was safety using all trans. The all trans as I mentioned in the human was primarily glucuronidation high affinity for glucuronidation pathway and it was primarily oxidized to metabolites that were still active and for total retinoids which includes the glucuronides. And we made comparisons between the minimally teratogenic doses in the animals and the clinical doses and dermal doses anticipated dermal doses in human. And these data are what we provided to FDA.

And then they said well what about extreme situations, so they hypothesize some extreme situations when a person is slathering the product all over body and not washing it off till the next day. We ran though so they could compare the margin of safety and so the conclusions then were that the models indicated that topical exposure resulted in 4 to 5 orders of magnitude lower internal exposure than the minimal terror to genetic doses regardless of which assumption you made about the measure of fetal exposure all trans or all the active metabolites. The FDA conduct did an internal evaluation of the PBPK model they concluded that it is one of my favorite senses I have ever seen. Internal exposure calculations are relatively insensitive to changes in the PBPK model which preserve correspondence with the experimental data. Another way of putting that is the if you believe the data, then you can believe the model prediction. So, they tried to sort of red team blue team. They tried to break the model and were unable to so that was the basis for them accepting the model conclusions. That is, I think, still a good way to describe model validation and acceptance. Alicia Paini will be talking in the next presentation about how currently the regulatory agencies are trying to deal with this question. The results of the exercise were that this result the FDA's concerns and they moved on towards labeling and the PBPK model is cited in the FDA approval of the NDA as a basis for relieving their concerns.

Thank you. Any questions? Thank you. Now I don't know how to get to the next slide. Probably have to—any suggestions?

Alicia Paini: Hi, this is Alicia.

A-V Staff: We will switch it for you.

Harvey J. Clewell, III: Oh great, yes. Okay.

So, I can go ahead and introduce the next speaker? What is the schedule? Oh, wow. If it is all right with Alicia, I think I would like to go ahead and let her start. Our next speaker is Alicia Paini from the European Center for Validation of Alternative Methods; it is part of the Joint Research Centre in Italy. Alicia has been a real ray of sunshine bringing PBPK modeling and metabolism understanding to ECVAM to help put together guidelines for the regulatory acceptance of physiologically based kinetic models. Actually, they dismissed the arguments between those who like physiologically based pharmacokinetic models, physiologically based toxicokinetic models, physiologically based biokinetic models, but they just got rid of the prefix

and went with kinetic. So, these guidelines have been under development for number of years under Alicia's leadership. They are now almost ready to come out and there is case studies, it is really quite a good deal of work. An international effort.

She will also talk about an example of a case study that she and I have both been involved in on applying PBPK modeling for route to route extrapolation of caffeine is one of the case studies for Cosmetics Europe efforts to develop new approaches for future risk assessments for cosmetics, and caffeine was used as an example of a compound with a good deal of data that could be used as a case study. In this case it is a matter of extrapolating from oral to dermal, I think. I will turn it over to Alicia.

OECD Guidance on the Characterization, Validation, and Reporting of Physiologically Based Kinetic (PBK) Models
Alicia Paini, European Commission's Joint Research Centre, Ispra, Italy (via webcast)

Paini: Okay. Harvey, can you hear me?

Clewell: Yes, yes certainly can.

Paini: Can you see the screen?

Clewell: Not yet, they're working on it.

A-V Staff: Alicia, if you can navigate to the next slide.

Paini: One second.

Clewell: There we go.

Paini: I can start?

Clewell: Yes, you can start. Thank you.

Paini: Yes. Okay, okay, good morning, everybody, I would really like to thank Harvey for the really kind introduction and also the organizers for allowing me to present the work currently ongoing at the OECD in the area of PBK modeling. I am actually very excited because this is actually the first time we are presenting this work and as stated in the slide, I have no conflict of interest and I also want to mention the document is not endorsed by the OECD and is still in draft. We have quite a consistent document to make a presentation for you today.

So, I would like just to start by saying we are all aware of the current paradigm shift in toxicology that is leading to the elimination of animal testing. We are trying to work concordantly to work new ways to assess chemicals for assessment. By using especially nonanimal *in vivo* data rely solely on alternatives and human data. This implies that we need, especially, to interpret the use of *in vitro* toxicity data in combination with biokinetic data that are covering the whole process of absorption, distribution, metabolism, and excretion. That are generated by *in silico* and *in vitro* methods for alternatives.

We know that this type of data can be integrated into the mathematical modeling. We know there is a wide portfolio of reliable mathematical models that can be used to this work one of them is actually a PBK model. Here as Harvey just mentioned, I would open this on the terminology. Actually, based on the definition that Harvey made in 2008, I have now tried to disseminate more the general term. I want to say that is always the same term so throughout my presentation you will hear a lot of PBK but in the next presentation you'll probably hear more of the other. It is always the same and they are all synonymous going back we say PBK models are one way to accurately integrate and use *in vitro* data, but we need guidance.

In order to actually have promote them there is credibility in the use of decision-making context. The basis of this statement is actually drawing back to several years of actual discussion and also document was published in 2015 on the commanding that we include more absorption, distribution, metabolism, excretion and other toxicokinetic data and model information. At least in the European side there is a few requirements on the regulatory frame umbrella for this type of information. This was actually followed by an expert work shaded in the end of [inaudible] establish more credibility of PBK models dealing more with uncertainty. Also, we looked, we were able to publish in 2017 results of the survey to retrieve information on PBK modeling using science and regulatory areas. The survey actually we received 93 replies. Here we really, with the information gathered by the experts in the workshop with tackling all the international people that are dealing with PBK modeling both as a model and other risk assessors, we were able to identify that there was really the need to develop guidance document for regulatory agency in reviewing acceptance of PBK model. So, this was really the basis of starting this project. If you would like to have more information on the survey you can read the paper, you can also look at the interactive map that re-created with all the information data at the link you can see on the slide.

Finally, I just want to mention that I was really intrigued when I saw that the chemical was actually took our work on the survey and actually made a news item out of it by really highlighting in red the regulatory agencies are reluctant to use mathematical models of organism. Triggered by these and also by all the other outputs that we receive, we were really aiming with OECD to try to find a guidance or trap this document in order to actually change this view of into regulatory area.

But let's say first that I would like to explain the difference between a typical PBK in the [inaudible] generational PBK model we highlighted in 2016. Typical on the one hand we have typical PBK models that are calibrated and evaluated relying on *in vivo* data. The model structure reflects the balance between the principles of parsimony where you have elements categorizing the system and also plausibility. That is actually attracting the of the physiological information. Then we know that we are in a kind of familiar uncertainty. We know the uncertainty by knowing of the application.

On the other hand, we have new generation PBK models for their development only using relying on *in vitro* or in cynical methods. These models, the model structures are an accurate understanding of the biology on the biochemistry and with this information, we actually generate more of familiar uncertainties. This new generation of PBK models have an increased productivity capability due to the inclusion of mechanistic assistance in this emerging new type of data. All this introduces really unfamiliar uncertainty. So new uncertainties. This is a new challenge as well for the risk assessor when they want to review these types of models. They are much more complex, and much more information needed also to kind of assess the parameters of the model.

So, all of this take into account, at the OECD, we went to write up the guidance document where PBK models are actually generated using also *in vivo* kinetic data are available for the validation of this model. The type of kind of regulatory application that we are envisioning is are actually mentioned in the table on the left and it goes from extrapolation to interpretation of biomonitoring data as well as *in vivo*, *in vitro* extrapolation and also why we are here wrapped around extrapolation. I would like very much to the document actually builds on existing guidances. It is not the first of the kind. There have been already several guidances and reaching out by experts on PBK modeling. The first one actually is from the U.S. EPA 2006. Until last year it was published by both the European Medicines Agency and the FDA. A guidance on the PBK model platform evaluation. We have actually had a really nice overview that we could draw upon and start building up our guidance. Several elements actually you will see later in the presentation are actually taken and have been adapted for the current scope of the guidance document.

So, going into the structure, it is actually quite simple. There is an introduction to main chapters. In the case studies. The first chapter is actually on the PBK workflow. I will show you all the steps that we report for this. In chapter 3 we have a regulatory assessment framework for the models. Finally, we have a list of case studies. We have around 12 case studies that are now currently being revised by the working group. I will take one of them as Harvey said as an example to show how we evaluated the model performance and how credible it is for us in the current scenario, in the current use.

So, going into the chapter 2. We have reports actually in scientific workflows that categorize dating of the model. We have five steps and these five steps are actually problem formulation and model conceptualization, model parameterization, solving the equations, and model validation. We give guidance for each step on what to do and what is needed. So, step by step. If we look at step one problem formulation is actually what we need so why do we use the model? What is the purpose? The model conceptualization is actually how to approach it so what are the minimal, what are the essential information that are compiling your PBK models, so which are the compartments needed that are expressing the mode of action of the chemicals? For instance, if we have metabolism, we would need the liver and also the metabolism. This is actually your drawing, a little bit your conceptual model. We then go to after this is done which are the information that you need to run the model.

So, you need to parameterize the model. So, we actually have two types of parameters. We have anatomical parameters that are actually looking at the bottle and we also have chemical specific parameters describing the absorption and distribution processes. These types of parameters to be measured also *in vitro* or estimated using physical chemical properties of the chemical such as the molecular ways, the partition coefficients, the air partition and in some cases, we also look at the biotransformation and metabolic rates. We also need, for instance, if we look at clearance then we need to extrapolate from an *in vitro* experiment to scale it up to *in vivo* situation. Also, we can account for metabolize by looking at the for metabolize formation using parameters. In the document, we are focusing on forming parameters of the model. The partition coefficient, the absorption of external barriers, active transport and clearance. For each one of them we are giving information on biological relevance, expiration of the parameter, classification of schemas, methods that are used to measure or predict the parameters. We give the uncertainty surrounding these types of parameters and in some case when available, we also try to help to show how to reduce the uncertainties.

We then go to step three. We are actually looking at how you solve the equation. After you have customized your model usually write down all your equations and then you, of course,

parameterize. I forgot to mention this. And then you need to translate the equation into a code. For this there is a kind, there are software packages that helps to simulate was algorithms and execute let's say all the differential, all the equation differential equations that are governing your PBK model. We have, sorry, we have done a really big effort in kind of compiling a list which is available in Madden et al. all information on software packages are available. And also, PBK model platforms where you do not really look, where you actually can input the input data and you get an output. In this paper and we also reported in the guidance document, there is a really long list of currently available software packages.

We then go to step four where we actually describe the model validation. So, we make terminology definition of validation, uncertainty and sensitivity. We also look at the sensitivity and uncertainty analysis and we also try to assess based on kind of a read across approach but we look at the predictive and the availability of data from data rich analog to inform the model that is actually built for a chemical that has no data. So, we try to push forward a kind of workflow where you need to understand how you can use the read across approach for using this type of information.

Finally, it is really important, the final step is actually the reporting. We know that following good modeling practice is really essential. It is in a transparent way we should report all the information used to build and how we apply the model. Of course, there are various templates available for this. We have developed one for this purpose for the guidance documents but there are also other events like the [inaudible] that have already proposed a nice harmonized template to report PBK modeling. All of this information can help to better assess the credibility of your model. They are all part of chapter 2 of the guidance document.

Then we go to chapter 3. Where actually report a framework that includes essentially to categories of consideration. In trying to address the of this model. For a given application. We are looking in the first is context and implementation which consider regulatory application and context of use. The second category is model validity which addresses content in the model. It shows up on the interested definition. We have also developed a reporting template. So, two tools that I will explain later. Reporting template and an evaluation check list. A bit further into the first chapter 3.

Looking under context and implementation, we have considered we need to consider several factors including evidence of the quality of the software qualification and peer review. We need to look at factors inferencing the degree of confidence in the model. For this, know that a pre-walk as it should always be that the PBK model equation or code should be reported I should be provided to ensure that the model actually does not have any mathematical errors, that the values that are reported of an input to the models are accurate and are also having the correct unit. That the mass balances always respect to and there is no numerical error in the execution of this whole algorithm when running the model. This is quite important information. We go into detail on why this is also needed.

Then in the second part, we have we can look at the model validity. Which is consisting. We kind of put up five main considerations. Five principles. Four of them we do not require *in vivo* data. For the biological beta we just look at model structure and parameters. This the writ theoretical basis is actually based on the model equation. Then there is the re-ability of input parameters. The input parameters I physically *in vitro* and *in silico* and carry new uncertainties. So, what we use adapting from the was actually this table you see on the right. We report for each parameter of the uncertainty versus the sensitivity analysis of the score of the sensitivity analysis. Once the parameter, the parameter will require a greater evaluation if it scores a high

uncertainty and has a high influence on the model while for a lower evaluation, it will when it will score lower on uncertainty and of course having a low and [inaudible] in the sensitivity and output of the model by sensitivity analysis.

We also mention and report information on sensitivity and uncertainty analysis in this chapter. We report two approaches that are currently used in PBK modeling. It is the one-time sensitivity analysis and the global sensitivity analysis. Both of them can have a big impact on model validity.

Finally, we have consideration of principles goodness of fit in pre-activity. It usually is actually based on *in vivo* data. So, we have created a PBK model evaluation toolbox where we first have a model reporting template as I just mentioned. With the metrics for reporting your uncertainty of the parameters based on the sensitivity analysis, and also, we have an evaluation checklist but also, we put together a kind of overall evaluation matrix. Also, this matrix is adopted from the of you guidance document. Going quickly through the model reporting template we can see that we have four different, we have different sections where you should fill in the person who wants to use the model the information that are provided on the model. On the model development let's say. This information actually following the principles a guidance document in chapter 2 and three. That we need to identify sub brings us to the modeling workflow. Then we identify the uncertainty. For each type of information for model structure and parameter of model input we should report all the model uncertainties. We also have information the software and if the coal is available or not for reviewing let's say.

Finally, the model has already done, the replication is very important. We then also on the right side I have put us not shot of the concise evaluation checklist that is built on two main parts. It actually comprised of 19 questions that need to be answered. It is actually a yes or no let's say. Checklist. Is quite easy to go through it. Usually need the templates to fill in the checklist once the template is filled and you can finalize the checklist. Then when I was doing this exercise of filling in and then using the checklist, I was coming out with the question what do I do now with my checklist? How can I interpret this? So, we have used this metrics for final kind of scoring and giving some conclusion to our assessment. We can say that if we have all let's say the information that are on the left side, we say that the model cannot be used because we have a low level of confidence. However, if we are scoring in the middle, it is not that the model is not reliable or not useful, it's just that we do not have enough confidence to use the data. We could actually use the supplemental information in our risk assessment. Well if we score high so we have all three on the right side of the metrics, we can actually be confident that our model is actually predicting correctly and we can actually say that we have a high confidence in the model and can use the information in the risk assessment process. Of course, here is a simplified version but we can have also that we score kind of in each different parts of the metrics. So, this we can adapt by saying we can have a reasonable confidence or not reasonable. We are still defining about all the components. I think this is the way forward of how you can conclude what we do with this checklist and with the template.

So, my final part of this presentation is actually taking into account using the caffeine case study that Harvey just mentioned. And putting first of all I will give you a bit of overview of the case study and then I will show you how I was able to evaluate this. So, we all know that the human health risk of exposure to a chemical can be characterized by risk characterization approach where you have human exposure level that are compared to establish limit values. Based on point of departure and then there divided by relevant assessment factors. On the other hand, we have a second approach which is using the margin of exposure which is actually the ratio of no NOEL or BMDL divided by the estimated human intake dose. These

approaches are not considering species and route dependent information. So, in the work that was carried out by [inaudible] Bessems, the MOI exposure was actually introduced in order to overcome this lack of not including kinetics in the assessment. So, with the margin of internal exposure, he tries to derive a systemic point of departure by PBK modeling as well as the human systemic exposure dose by PBK modeling.

The approach following this approach, it was characterized using caffeine as an example and the risk of caffeine exposure and dermal products. Actually, we looked and case studies for oral dermal extrapolation since caffeine is that ingredient in cosmetics. For which we have oral animal toxicity data. What was done was that the PBK model was developed for caffeine to convert the chosen two internal dose metrics under the current and the oral human model was used to predict internal dose metrics and was actually calibrated and validated. Then to this model, we expended it extended it using with a dermal department to accommodate a few realistic exposure scenarios. Cut internal exposure for cosmetics and it was actually built taking into account users to simulate human dermal and attrition.

The results were that using direct oral and the human dermal we were able to compare in terms of the resulting margin of internal exposure using the internal metrics and C_{max}. This a little bit what was done in this work I took the information that were reported in the paper. I tried to populate the template that was from the document. We had information that the code was developed, the equations were reported. The bottle calls and all. The model assumptions were reported. The model concept was also part of it. Also, which parameters were used to parameterize and to validate the models. As you can see here, I just want to show you that this information was available. There was a lot of assumptions leading to a lot of uncertainties. Also, the PBK models was very complex especially as you look at dermal. Even hair follicles were put in. What is really, really meticulous, was really, really well set up. It is quite difficult to actually give a hard-core if you know all the uncertainties behind.

So, for this, for this PBK model, we actually, I actually scored a stand of confidence so that information can be used in the risk assessment only as a supporting document. In order to be really able to use it, the model should be reevaluated let's say. So, actually this was done as Harvey mentioned in a new case study that was led by what was pushed forward by the Cosmetics Europe as an IATA, which stands for integrated approaches for testing and assessment. Here we have actually an extensive revision of the model was done. There was a lot of simplification and it was recorded in Berkeley Madonna and new data was used to parameterize the model. There was conflicting of the previous model, we saw that the model improve and now we can say that we scored a more reasonable, we have a reasonable confidence can be used in the IATA concept in the risk assessment area.

So, my take-home, is the take-home message that I would like to give is the guidance and the PBK toolbox are actually I hope expected to per note the use of this model in the regulatory area. We hope to provide a harmonized framework not only to correct and update the model but also to facilitate the dialogue between the developers and the end user for the risk assessment. So, the target audience of this guidance is actually model developer and risk assessors. Which have a total different view as you can see in this figure. It really captures what a modeler or a toxicologist thinks like. We know a risk assessor or risk manager likes a yes or no for simplicity. I really like to try to facilitate the dialogue between the two and I hope that the guidance document will help in making this effort coming real.

So, with this, I would like to acknowledge all the people that have been working with me on the OECD PBK model guidance, especially [inaudible] Sachana from OECD that has been

coordinating the effort, and [inaudible] Worth from JRC, my colleague, and. [inaudible] Tan who is also leading leading together with Andrew this work. And also, all the people involved that are actually currently providing the second version of the document. We hope to submit to OC ED in April of this year. On the other hand, I would like also to thank the colleagues of the Cosmetics Europe case study. They have agreed that I could share some insight into the model information. However, this has to still be approved as an IATA so no details I could share the present moment. Especially I would like also to thank the people that were involved in the 2017 survey. With this I would like to thank you for the attention and if you have any questions, I can answer them now or we can keep them for the final discussion later.

Clewell: Thank you, Alicia, was a very nice presentation. I really think that the guidelines that OECD is developing are going to play an important role in moving modeling from the *in vivo* base to *in vitro* and silicone-based in terms of the regulatory acceptance. I think that is a very important step.

Are there any questions for Alicia, kind of specific questions to her talk? Are there any from the web? No? Okay. Okay. Alicia, I have a question for you. I love the coining the new phrase margin of internal exposure and I think that publication has already had some impact, particularly for the OECD case studies. I'm wondering do you think that is going to become a well-known or standard term in regulatory agencies? Do you think it is going to become kind of an accepted idea?

Paini: I hope. I think it would make [inaudible] Bessems very happy. I was actually discussing with him two weeks ago about this terminology. I think once we have really the okay from the risk assessor on the PBK modeling side that they're happy and they are not reluctant in using them, I think this check terminology will also be more and more used because then we can really go inside into the internal exposure. I envision that yes, this terminology can be really be in the next phase for risk assessors to be used in the regulatory context. To keep it brief.

Clewell: Yes, well, that is good. I think this probably will be something that Europe will be the first to deal with. I hope that it will gain some traction there. I did want to mention Alicia referred to Berkeley Madonna in her talk. I personally believe that Berkeley Madonna is the modeling platform of choice currently for exploratory PBK modeling. It is easy to use, it was developed by a couple of professors at University of California Berkeley. That's the basis for the name; they were being cute. It uses a continuous simulation language convention that allows you to more easily read and develop code. I highly recommend it. It is cheap too, but it is between $100 for a student license, perpetual license, and about $400 for a company license.

So, anyway, the language that we used in the early years of PPBK modeling and risk assessment was ACSL and is no longer available. It is sad but I would not have been anywhere near as productive if I had to do all the modeling. I would have used Fortran. Now it is counterintuitive, but everybody is going to R. It is not a good language. It is about as good as Fortran. You can write anything you want because it is unstructured, from a programmer's viewpoint it is terrible. But it just happens to be the one that is around. And free. Everybody seems to be using it. I think that is really a shame because writing a model in R and reading someone else's model in R is horrible and I do not consider the [inaudible] time environment to be acceptable. Anyway, just my two cents is try Berkeley Madonna.

What we do at Ramboll is I run in Berkeley Madonna and my associate runs in R and then we check each other. And very often, well, not very often, okay, I'll be honest, but every once in a while I find out that is done because I cannot reproduce it but it turns out oh yes, one of the

scripts, there was just a little error in the script, and as far as I'm concerned, there is no such thing as a little error in the script. It is just wrong. If I wasn't doing Berkeley Madonna, we would be putting up the wrong answer. But anyway, enough said.

We're still ahead of time, but I think we are close enough that we can go on to the next presentation. An old friend, and I stress the old, of mine, Dr. John Lipscomb, is going to cover a very important area of *in vitro* to *in vivo* extrapolation of metabolism. It's one of the fundamental concepts that needs to be understood in modern risk assessment if you're using [inaudible] or Berkeley Madonna or anything. The one parameter that is the hardest to find data for is metabolism, the best way to do it in today's environment is *in vitro* studies, then you can put together the model parts from that with QSAR and literature. So, that is a very important part. John is in a good position to talk about it.

He started out as an NCTR, I did not know that; I met him in the Air Force when he was doing metabolism studies and I was doing PBK modeling. He went on to join the EPA, had a distinguished career at EPA Cincinnati. Now he is semi-retired back in Arkansas, working at oh I forgot the name, CTEH, an emergency response company that supports emergency response situations. John will talk about *in vitro* to *in vivo* extrapolation. John?

In Vitro to *In Vivo* Extrapolation of Metabolism Data to Support Physiologically Based Modeling for Route-to-Route Extrapolation John C. Lipscomb, Center for Toxicology & Environmental Health, North Little Rock, AR

Thank you, Dr. Clewell. I appreciate the warm invitation. Started my career in down at NCTR in Jefferson, Arkansas April the 15th, 1984. It is good to be back at FDA here again. Whether that makes me old or not is up to you. I've got an audacious title. *In vitro* and *In Vivo* Extrapolation of Metabolism Data to Support Physiologically Based Modeling for Route-to-Route Extrapolation. That being the case, you can probably imagine is going to be a fairly in-depth topic indeed, it may be a little more technical than those before.

You have heard the need for route to route extrapolation. You have heard some discussions about what is physiologically based modeling and how do we know when a physiologically based or of PBK model is good? When we say good when we think about is fit for purpose for that is important in the area of health risk assessment because there are many data sets out there including PBK models that have been developed for other purposes but they're not exactly fit for what we need to apply them to. That is the area where we will be now. Will be talking about some *in vitro* systems at the end of the talk which really is the subject of the talking why am here in the first place but I was given some remarkable opportunities in the U.S. Air Force where I was given the responsibility and really the privilege of working with some of the finest people in the world to develop some of these *in vitro* data sets that have been used in physiologically based models that have been used by the U.S. EPA. Subsequently to base regulatory decisions.

There is not only a quote academic field it is very applied and practical field. We talk about toxicology we think about the dose-response relationships but when we think about the movement of the chemical from the body into the target tissues so that they will produce the effect we have to think about the continuum of the movement of the chemical from the environment through the absorption barriers into the body to produce a target tissue

concentration or target organ concentration that is then and only then responsible for the production of the biological response.

So, what is this target tissue concentration or TTC that are we going to be focusing on as the basis for route to route extrapolation and indeed, this was the basis for the important paper that Alicia showed from the colleagues about moving the margin of exposure comparison from the animal point of departure and the human exposure to comparing the animal internal dose at the point of did archer to the internal dose in the human under the conditions of human exposure. When we think about extrapolation, I usually think just from my own mindset of moving from oral stage to inhalation exposure. The reason I do that is because I come from the world of mostly oral toxicity and inhalation exposures are important for the human.

Conducting inhalation exposure in humans for the purpose of dose responses preclude conducting inhalation studies in humans is a very difficult thing to do. Conducting insulation studies and wrote this is a very expensive thing to do. We typically use oral dose from animals to evaluate exposures and responses from other risk, from other exposure routes in and of the species. I will show you one that dates back to 2000 which I think is still one of the finest examples both across species in route to route extrapolation within a U.S. FDA's inventory per that is fun that Harvey mentioned before it is the assessment for vinyl chloride. When we have data from one route like the oral route you see on the slide but we wish to determine whether those data can be used for the inhalation route but where we express toxicity city in terms of not just the dose-response relationship up of the target tissue response relationship. We can see whether we are going to be successful in our route to route extrapolation or not.

So, what we have response from the, those two response curves should overlay when we express the dose, not only at the implied dose in the external dose on the left but the target tissue dose on the right. If we do the route to route extrapolation and they do not align, if we have data somehow indicate whether they align or not we are in good shape it if they do not align, we know we have got a problem. The goal of this is to harmonize the X axis for the dose relationship. Some basics Harvey already mentioned: don’t do to route to route extrapolation for portal of entry tissues. There is generally a no-no. The site of damages in the upper respiratory tract the nasal passages or the tracheobronchial region or the pulmonary reason, usually it's going to be very difficult to extrapolate from the oral route to that dose metric just because you have the impact of potentially reactive chemicals and physical chemical properties impacting the toxicity. We need to have not only an understanding of what is the tissue, what is the portal of entry, but we need to have some good dose response data.

Obviously, we have to have confidence as you just heard Alicia talk about in the [inaudible] model. We're going to construct a virtual parallelogram that has been used in the area of *in vitro in vivo* extrapolation for a long time. Here were going to use it from route to route. We have to have dose-response data, toxicity data from one route from my perspective the oral rapid we need to have confidence in the data and we need to have a formal chemical model that reflects not only the chemical and properties but the organism that we will be using. To do the route to route extrapolation we have to use the same models for one route as the other route. We cannot change parameters, we cannot change tissue volumes, we cannot change tissue flows, we cannot change partition coefficients, we cannot change metabolic. The only thing we can changes the way the chemical enters the body. We can change it to an oral or dermal, or even an IV if we need to. That is the only thing that is change. We use exactly the same model. We use dose metrics. I talked about target tissue concentration, that is one example of a dosimetric and I will show you where others are.

So, we want to translate the exposure from an external dose to an internal concentration to get that. There is the graphic. If we want to stop at the top left were going to think about toxicity and toxicokinetic data from the oral route. Then we are going to think about toxicokinetic data and risk assessment for inhalation rate for the chemical comes in and said is exposed via ingestion. It gets into the systemic circulation, it's distributed to bodies, we get the processes we get the distribution to [inaudible] and that we get a response. Here we can think about mode of action, which is another important concept. What is it in and risk assessment?

We need to know three things to characterize a mode of action for these purposes. We need to know whether metabolism is leading us to understand whether it is metabolized that we need to be looking at. If it is a metabolite, we need to consider what forms of metabolite, what is the enzyme, where is it located. This bit of knowledge leads us towards a selection of the and propria *in vitro* system to use. So, if we know what tissue and whether it is the parent of the metabolite then we can begin to think a little more technically about what is the best dose metric. Well, a dose metric I mentioned can be a target tissue concentration, there are several other potential dose metrics that kinetic modelers and others have already thought about. Some of the other dose metrics might be one that we call AML, the amount metabolized in the liver and you will see that in the example. How much is the metabolized form of unit mass for level? Another one is the formation; does it proceed at such a rate that it depletes the normal mechanisms in the river? What is the metabolic pathway, do you have a multi-step process? Is it a concentration? Is it a maximal concentration? It is a threshold or is it a normalized exposure like the area under the concentration times level. This is what motive action is in the formation of modeling?

Once we have those six determined, we can choose the appropriate dose metric the dose-response curve to show the relationship between the dose metrics in the response. We have that done for the oral route, we pop that over into the inhalation route. We quote run the inhalation model backwards to identify the concentration that would produce the same level of the dose metric in the oral route. That is the fundamentals of route to route extrapolation. It seems simple.

Let's get into a just a little bit more and see where we are. To do the route to route extrapolation, we are going to need two logical frameworks. The first framework is that pf physiologically based pharmacokinetic modeling you've already heard about. This particular graphic shows the potential for exposures via two different pathways. The first is would be an inhalation exposure where the chemical goes into the gas exchange long and then enters the systemic circulation to be perfused all the tissues. The second arrow of the bottom goes to the ingestion route for the chemicals there would be orally encountered into the stomach via G.I. tract to the liver for the first half. And then to the different tissues of the body there is a cardiopulmonary circulation for the red star indicates that we are going to be looking in the example at a chemical that produces toxicity in the liver. The star is simply there at this point to indicate that is the target tissue that's what we are going to be looking at. Metabolism is covered by about four parameters in PBK models.

The QL is flow to the liver; we cannot have metabolism in the liver because the substance doesn't get there. Liver blood partition coefficient covers the movement of the chemical from the blood into the liver and is a passive measure based mostly on water and lipid content and optimal water partition chemical. The two metabolic constants KM and Vmax are usually determined because most of the chemicals I have dealt with at least are saturable and the metabolism is saturable. We need to make sure that we have the model adequately developed we are going to use the model for both the of the extrapolation procedures both extrapolating

the point of departure, the POD value, from the oral route to a target tissue concentration and the extrapolation of the target tissue concentration through the inhalation model to the inhalation exposure. So, once we know all these things, we can get to that.

A little bit about enzyme content and the quantity of the enzyme. If we know that the parent chemical is the toxicologically active chemical species then we can use quote a generic system to evaluate the degradation of the parent compound because we are only interested in what the parent compact is, we are not interested so much in what the different metabolites are. We can focus on the parent compound. There we can use a wholesale-based system either as isolated or in some cases, tissue slices to determine the metabolic rate constants that we need but the rate constants will be expressed in terms of disappearance of the parent compound. We need to remember anatomy, biochemistry, cellular biochemistry, and physiology, and we need always work within the context of the intact body because while we can control things very well in the *in vitro* setting, like substrate concentrations, times of incubations, those are not always either so well-controlled are so well known in the body, so we need to always impose the constraints of physiology, anatomy, and biochemistry on the *in vitro* findings that we can develop.

Now let's look at what the parallelogram approach looks like for a route to route extrapolation. Here were going to be, this graphic shows the extrapolation of oral point of departure to an inhalation equivalent. If we were doing a cross species extrapolation using PBK models that would be the human equivalent dose of the human but here we are working with one species. Say for example the human. So, if you have the dose that we are interested in and the dose has been identified via the oral route, call it the point of departure. So we'll simulate exposure to that point of departure dose on the oral route there via the ingestion. We use the model to predict what would be the target tissue concentration in the liver for that exposure and then we will identify and fix that target tissue concentration.

We will then operate the inhalation to simulate determine between target tissue concentration and exposure and when we input the target value for target tissue concentration then we can identify the inhalation equivalent to that. That is how that works. The text at the bottom of is a reminder to reinforce to you that here I am using target tissue concentration dose metric interchangeable, here, the dose metric that we have chosen concentration and that's not always going to be the case. It may be a metabolic looks pathway but here in this example it is the amount of the metabolism form.

The parallelogram. We have three points known on the parallelogram one unknown. The top left would be the oral toxicology where we picked the point of departure. The bottom left point would be the oral toxicology tax where we translate the oral departure to a dose metric in the bottom right point would be the inhalation talks a code phonetics where we extrapolate the target tissue concentration to the upper right point, the inhalation equivalent to the oral dose. Those are the four points of the parallelogram and when we have those four points, we can work through what we need to do.

Vinyl chloride was published by the US EPA on its integrated risk information system in 2000. I have adapted the analysis for this presentation to focus only on the route to route extrapolation. The original analysis was done to assess the human inhalation for vinyl chloride. We had very few data from human exposure they were still from human exposures than we had a fairly rich animal oral database. We had a relatively sparse animal inhalation database, so we knew the dose response from the oral route in animals and we needed to predict the response from an inhalation exposure. Again, for the purpose of this presentation focusing only

on the route to route extrapolation but if you wish to see more of it you certainly can I looking in either the IRIS database or chapter written by [inaubdible] in the reference section at the end of this presentation.

We knew from the animal exposure that liver was going to be the target. We also had some human epidemiology studies that let us know that the liver is also going to be the target in the humans. That's where we are starting. We know for vinyl chloride the target tissue is going to be the liver so that's where we're going after is the target tissue. We also know for vinyl chloride it was the metabolite not [inaudible] so we know two things already. We know the animal model shows the liver and the human data corresponded and we focused on the liver is the target tissue and we know it's metabolized. The only other thing we need to understand is what are the units of extrapolation in liver. We know we're going focus on the liver, so we know the denominator of whatever dose metric is, is going to be in liver to correspond to what we know about the target tissue and the concentration. There is a series of investigations led ultimately to the publication and conclusion that you see here from Andersen and colleagues some of who are in the room. The most appropriate pharmacokinetic dose metric for reactive metabolite is the total amount of the metabolite generator divided by the volume of the tissue into which it is produced. Here we go. Metabolite produced in the liver divided by the volume of the liver to get to the concentration of the metabolite liver which in code is written as AML. We are going to use AML. We will talk about AML here in the generic sense as target tissue concentration because it is the amount divided by volume and that's a concentration.

Let's go back and talk about the parallelogram approach. We have toxicology data on the left and that's the point of departure was 0.13 milligrams per kilogram per day. That's the external dose or ingested dose consumed by the rat that produces the liver effects that we've chosen as the point of departure. We use the animal oral toxicokinetic model to determine what the dose metric associated with 0.13 milligrams per kilograms per day and what we find is that corresponds to a target tissue concentration of three milligrams per liter of liver. That's going to be shown in subsequent slides in the market is going to be for that is going to be the red triangle. When you see that red triangle think of three milligrams per liter of liver. That's translated to the target tissue concentration internal dose chosen at the point of departure. This would be the dose exposure in the humans for the point of departure that would've been used probably in the estimation of margin exposure so in context of that's, that's where we are with this the modeling also shows that there's a linear relationship the tween exposure of target tissue knowingness gives us an increased—why is that? That's because if we’re off just a little bit we are not near the point of reflection and not near saturation and were not near a point of gross uncertainty but we are in the linear range so all things should be fairly good even if were off just a little bit.

At this point, what are we going to do? I been talking about doing the route to route extrapolation and using a [inaudible] but why can't we do this in humans? I’ve already said many of the reasons why. But what can we do in humans? We can and we have for a long time used very high-quality specimens from liver biopsies and organ donations recipient use these very well and development of *in vitro* protocols and studies many of which have led to some of the findings presented here. It is important to consider the quality of the human tissues that we have for investigation. The organ procurement system is highly controlled for good reason it has a good outcome there are number of situations for the donor that preclude the use of that organ in an actual transplant. Some of which are easy to understand, and some are policy driven and we will not get into those, but they do not impact the quality of the tissue.

An example might be we have a good match between a donor and a recipient from, from a biochemical standpoint but human anatomy is a fickle thing, sometimes the VAT sketcher is different and if the vascular in the donor liver does not match the vasculature of connecting the liver to the body of the recipient then that is a no go.

Another example is presence in the prison population typically what we used to see and I don't know if this is the case now but the regulations were if a donor had been included in the prison population that meant there's the potentially uncontrolled exposure to pathogens etc. so that organ would usually not be transplanted into a recipient. If the organ was still completely viable and those working *in vitro* often got tissues like this into our laboratories to work with so those we were entirely grateful. Human data we can use so we plop it into the *in vitro* parallelogram but use it in the context of route to route extrapolation. That's what we did. It is important that we think about those things when we do that.

When we completed the route to route extrapolation based on some of the *in vitro* extrapolation shown in the final slide here on the vinyl chloride example shows the bottom left the oral part of departure 0.13 milligrams per kilogram per day translated via oral model to tissue concentration three milligrams of per liter of liver to run the inhalation PBPK model—to be equivalent to the oral part of departure 2.5 milligrams per cubic leader of expired air. That completes the route to route extrapolation that was done and those two exposures, the 0.13 milligrams kilograms per day and the 2.5 milligrams per cubic meter, are toxicologically equivalent. If we can say that with some certainty and move on to what is it that we are going to do. We are going to use our human tissue, but we also need to keep in mind the linear rate of metabolism and work in the linear range where we have a greater degree of confidence than we would otherwise [inaudible]. There is a lot of feedback. Could someone mute the external microphone? Sounds like a printer in operation.

A-V Staff: We are looking for whoever that is.

John C. Lipscomb: I am afraid it is distracting to participants online.

A-V Staff: Whoever that is can you please mute your phone or whatever that is, please?

Lipscomb: Thank you. So, certainty. Let's think about whether the compound is toxic as the parent or metabolite and whether we have some errors somewhere. If we know the parent compound is toxic and we know metabolism in this hypothetical example is not connected to vinyl chloride by the way might even be nearly completed at 95% or virtually complete at 99%, then the difference in risk is about fivefold. If we are off and it's 95% if metabolism is truly 95%, we predict 99% the difference they are in the remaining parent between 1% and 4% is about fivefold. That's a big error. However, when metabolism results, results in a bioactivation process and if metabolism is fairly completed either 95 or 99% cotton what we see is a difference in 1% versus 5% or 99% parent versus 95%. We see the difference in the amount of the form metabolite either 99% formed or 95% formed is only about 5%. This is a situation we have with vinyl chloride. If we are off just a little bit with the metabolism, then we are still not going to impact risk a lot. That gets back to Alicia's earlier slide about what confidence we can have in the model and how do we know we can have those levels of competence. This is one example of how we can poster competence.

Let's look a little bit more about another bioactive compound trichloroethylene metabolism processes oxidation is done via [inaudible] oxidative are in the microsomal conjugate [inaudible] to completely distinct places in the cell. We have two different places where we

could be looking at for the purpose of this example, we looked at cytochrome P450-dependent metabolism which was responsible for the formation of [inaudible] acid. Those are two the toxicologically active metabolites, we are focusing on the risk associated with the metabolite, so we focused on the P450-dependent oxidation of trichloroethylene and we knew that was an enzyme expressed on the endoplasmic reticulum of the cell and isolated microsomal protein. Or the microsomes. We studied microsomal protein where we looked at trichloroethylene metabolism. First thing we did was characterize in rats, mice and humans *in vitro* and mice had the highest metabolic followed by rat and trailed at some distance by humans. Wanted to think a little bit more about why that was and we began to look at some marker substrates and what we found in some of the original evaluations there on the bottom left where the metabolism of trichloroethylene on the Y axis was correlated with the metabolism of [inaudible] which was a marker for cytochrome P450 2E1. The [inaudible] was metabolized by CYP2E1, so is TCE or at least they correlated very well so had a pretty good idea that we wanted to focus on CYP 2E1.

We will need to quantify the enzyme so working with Dr. John [inaudible] down at CDC NIOSH in Cincinnati he developed an ELISA method which was sensitive microsomal protein isolated from the liver a for species. [Inaudible] is a small fish in research, humans, rats, and mice and what we found the Vmax for TCE in numeric presented their correlated well with the protein—CYP2E1 to the oxidative so then we needed to think about where CYP2E1 so we ran a series of, of CYP2E1 to quantify CYP2E1 in the liver divided by microsomal protein and then if we needed to do something different we could have also evaluated metabolism in the [inaudible] studies available that demonstrated the amount of hepatocytes present per gram of liver. All of these as well as liver mass and body mass are necessary to translate the metabolic through units that would be useful in PBPK that is that one is the amount of [inaudible] data set to amount of and data set three amounts of [inaudible]. Those were mathematically combined which you'll see at the upper right slide oxidize per minute per that will still show you on the next flight to appropriate units to drop into the model. Focusing on the right side of that slide would be a lot easier for you. Show the actual data in the mathematical extrapolate your mathematically based extrapolation of the turnover number all the way through starting with [inaudible] through the concentration of CYP2E1 and microsomal—ultimately we get correct mass in time the unit for 17 milligrams per hour per kilogram body mass and this is the unit that was put into the TCE model. We are focusing mostly on Vmax here. We are going to focus on KM and that's because that area is still evolving. Most of the metabolic rates that we see occur in exposed humans that concentration far below KM so it's really not much of a player. What you need to consider is when we use the [inaudible] kinetic models that we express sub in the same volume that's the bottom line for that.

KM can be put directly into the model either as substrate concentration used in combination or could be as concentration that was in our example the microsomes within the microsomal and incubation hepatocytes if we did that one thing about the biology of the Intech organism we can see there would be potential wide differences in the amount of chemical metabolized *in vitro*. Those differences may or may not be demonstrated *in vivo* a lot of that depends on delivery of the substrate to the liver. Delivery of the substrate is determined by at least two things, the flow of blood to the liver and [inaudible] movement of chemical from the blood to the liver. Trichloroethylene is one of many substances that we see that has a metabolism that kinetic model described as flow limited. That means the metabolism is only limited by the rate in which substrate can be delivered to the liver.

Under these cases it is important to remember that increases in the enzyme content of the liver will not result in increases in the bile activations by our risk standpoint enzyme in the liver will

not cause more risk and why's this? It's because even basal conditions the amount and activity of the enzyme present in the liver is already sufficient to metabolize more than the amount of substrate that is available for delivery. Think about the physiological content. If we know what the Vmax value is, it may be unlikely that humans will ever be exposed to concentrations that would get to that level because using TCE as an example those vapors if inhaled would produce unconsciousness a situation that would not be tolerated. It might result in death long before we reach saturation of metabolism.

That's important to consider when we do these *in vitro* extrapolations that they have to be interpreted within the context of the whole body. That's what this whole presentation has been about. We talked about route to route extrapolation and recapitulate what we heard earlier. We got a little into pharmacokinetics and constraints of the body and have shown you the mathematical process and generic formula for the process can be used for virtually any enzyme and it's an important to consider from the get go we have to understand whether it's apparent chemical or metabolite and sometimes what the metabolite is what the target tissue is and what's the unit of measurement for the dose metric that were going to use when we know those things we are set on a firm foundation to use physiological pharmacokinetics and metabolic rates of metabolism extrapolated from authentic human tissue put into the models to drive them. With that I will conclude my speech and you can read the summaries. Basically, we need to know let's do this within the context of intact system. Thank you.

Clewell: Thank you, John, that was very nice. Actually that reminds me one of the things that I discovered along the way was talking with people in Pharma when you're in the environmental world, always starts from mixed premises. Drugs are slowly metabolized or else they would not be druggable. If they are to rapidly metabolized, you would have to take one every hour. Environmental chemicals are typically, for these kinds of chemicals, very rapidly metabolized. The implication that one is flow limited, metabolism is John describe as [inaudible] vinyl chloride and other chemicals, tends to be flow limited, whereas drugs that's not the case. Another difference that comes from the same reason is a drug companies, well for pharmaceuticals, it's generally assumed only the fraction that's found in the blood is available to metabolism and that works most of the time and then when it does not work they say that chemical is the exception, but actually in the environmental modeling for not only environmental contaminants but many cosmetics, it actually is more often the case that the chemical is totally available from the blood because the binding is reversible and the extraction is so high in the liver that chemical is pulled off of the [inaudible] during its transit of the liver in the blood. There is a very different kind of assumption between modeling of pharmaceuticals and modeling of environmental chemicals.

We are right on time. We will get back together at 10:40 and John Troutman will give us the next presentation.

Break

Clewell: I would like to welcome everyone back. We are ready for final two speakers and then the panel discussion. Our next speaker is a senior scientist at Procter & Gamble. He is been in Procter & Gamble for over 20 years after spending a number of years in the pharmaceutical industry and he has a good deal of experience in both PBPK and incorporation of metabolism data, and application of these kind of modeling applications in route to route and other kinds of risk assessment implications. He is going to talk about internal margin of exposure concept

and application for oral to the dermal to phenoxyethanol and more work being done as OECD case study by the cosmetics industry. John?

Determination of an Internal Margin of Exposure between Rodent Oral and Human Dermal Exposures to Phenoxyethanol Using Physiologically Based Modeling
John Troutman, Procter and Gamble, Cincinnati, OH

Thank you for the kind introduction. I would like to take a minute to think the organizers to share me allow me to share to speak physiologically based models in risk assessment. The title of my slide is Determination of an Internal Margin of Exposure between Rodent Oral and Human Dermal Exposure to Phenoxyethanol Using Physiologically Based Modeling. I have no conflict of interest to declare other than I am an employee at Procter & Gamble and technologies in the presentation may be used in the safety evaluation of P&G products.

A brief overview of my talk the previous speakers have done an excellent job of covering the basic concepts. I have a few slides that are addressing concepts and risk assessment and also a few slides on the fundamentals of absorption, distribution, metabolism, and excretion, and then we will move into the case study specifically on the work we did to develop the kinetic model for phenoxyethanol and how it was applied to reduce the uncertainty factors in the risk assessment. This work was a collaborative effort between industry and government. We had investigators from Procter & Gamble and Dow Chemical as well as Dr. Fisher at the NCTR who contributed work which led to a publication in more detail of this work with phenoxyethanol.

This slide is a little redundant however we are all familiar with you have an external exposure and it can lead to nonspecific unwanted effects internally that can ultimately lead to a response that could be measured by observation or measure by sampling matrices to look for deleterious effects. What we are after is rather than basing this assessment on external dose in some cases could be misleading or uninformative will want to try to target and understand what the internal concentrations are of a given compound within the site of toxicity or organ so we can better characterize the nature of that toxicity and understand the exposure as you increase the exposure level and extrapolate from a given route in between routes and between species you can account for these kinetic differences that drive the internal concentrations within the organ of toxicity. Pharmacokinetics are used to relate with external composure relative to the internal concentration and dynamics piece relates to tissue dose relative to the response.

This is again review in simple terms risk is define by hazard data obtained from talk studies and exposure. For noncancer endpoints, safe levels are determined by dividing a threshold level point of departure or benchmark dose level by the uncertainty factors that are assumed to be held protective. These uncertainty factors can be conservative, or they are so because more or less uncharacterized for and certainly in extrapolating between dose levels between species and different doubts routes or within a given population.

The default uncertainty factor 100 is divided or subdivided into inner species differences and inter-individual differences further subdivided into toxicokinetic and toxicodynamic components. These are used to address some of the differences they could occur between extrapolation between animal relative to human and when data is available from the animal studies or in humans, the pharmacokinetic models or predictions can be used to refine and

replace default uncertainty factors with chemical specific adjustment factors. In this case highlighting toxicokinetic factory could be used and it can again be adjusted.

This slide is representative of a concentration profile of a compound administered time equal to zero in this case in the shape of the curve in concentrations that were measured in the samples are specifically influenced by the kinetic processes of absorption distribution algorithm and [inaudible] what are the underlying mechanisms that result in lead into the concentration in the profiles that are observed and you can see that absorption is one piece with rising concentration it reaches a maximum which is known as C_{max} and indicated by T_{max} and followed by decreasing concentration over time and of course all these processes of absorption distribution [inaudible] some occur at a higher rate as others but it's really the understanding of the shape of this curve looks like in understanding what the specific concentration wallowing exposure would have to really influence what the internal concentrations would look like in how those internal concentrations could be different given route of exposure or dose level. Really is exposure level cut frequency in which the exposure occurs at a given site, what is the route of entry and those are all major determinations of what the exposure would be and ultimately its activity. If we can understand what these differences are between the test species and humans, we can quantify them and put them into a model to better understand and characterize found in the uncertainty factors.

The slide is a simple box analogy in the four quadrants you have amount of chemical at site of absorption, the rate and extent it brings the compound from that into the body and distribute into other tissues and would be available for elimination from the body either through metabolism or the formation of metabolites or excretion which would be the removal of the parent compound unchanged through other pathways and that could be biliary urinary exhaled eliminated through sweat. Understanding the pathways are critically important in getting a conceptual understanding of the molecule and how you would want to approach the development of a biokinetic model to inform what these processes would be and how to go about generating the data you need and parameterize for your simulation.

This is a more physiological or animal comical presentation the previous live. Here we are thinking again about the relative sites of exposure how the exposure scenarios would be different following an oral ingestion and it could be an oral—drinking water over a longer. Of time or through a feeding study. We want to think of the nature of that occurs and similarly skin how much is applied what area of the body is the removal of the product or compound at the specific time or is it a [inaudible] exposure.

From that we can them form what are the relative rates of absorption what's the rate and it extent it's going to reach that mass that supply—systemic circulation and what are the processes that would influence what the internal concentrations would be for the oral route obviously it would be the ability to cross the gastrointestinal tract that could increase systemic by gut micro [inaudible] parasites which could reduce the concentration that would reach the liver and then the liver metabolism biliary excretion in those processes could influence what the internal systemic concentrations would be. In this slide I have metabolism separated in its own box because we think about metabolism is occurring in the liver and ultimately a primary organ of metabolism but metabolism could occur in other organ so we want to be open to the idea there could be metabolic activity by other [inaudible] concentration understand. That we have elimination by different routes, the fecal route excretion or [inaudible]. Again, this is redundant, but these models are being increasingly used and accepted as approaches that can predict the concentration of chemicals drugs or active metabolites and use of this information can improve our scientific basis for human health risk assessments. Specifically,

for this we are focusing on refinement of default uncertainty factors specifically inner species differences known to occur between rats versus rodent species and humans and also use the data to better define the enter individual differences occur within a given population. You have pediatric populations or geriatric populations that could have different compromised organ systems and we can also use it to extrapolate between different routes and different dose levels.

Moving on to the characterization work we did for phenoxyethanol for the risk assessment, the doses of 400 meg per gig day toxic effects include hepatotoxicity renal toxicity and hemolysis. Data available from the rodent studies showed and again these were rodent studies conducted by different routes of exposure somewhere dermal summer oral and some of the oral were bulletin somewhere drinking water. The profiles led to different NOEL values and low L values that somewhat overlap. We have NOEL range 82 1875 and LOEL from 400 to 3700 we really needed to think about why is differences in the overlapping dose metrics would occur from this analysis a rat NOEL is 369 from critical drinking water studies identified as the appropriate repeat dose using benchmark dose modeling based on liver effects following [inaudible]. The objectives were then to use biokinetic models to quantify the impact of species differences in metabolism which are known to occur and also to understand the influence differences between the routes of exposure on the observed effects and explain what they appear inconsistent across studies. Second was to use the estimated internal exposure to reduce the uncertainties in the kinetic differences between the species and refine and improve the overall human health risk assessment through application of PBPK modeling.

Fortunately, phenoxyethanol has been well-characterized in mammals. We know it's rapidly metabolized following oral and dermal absorption is mediated through alcohol and [inaudible] formation of the [inaudible] 75 to hundred percent of orally ingested or dermally is absorbed dose is excreted in urine as [inaudible]. The asset metabolite can be used as a relevant biomarker for phenoxyethanol exposure. Cumulative amounts in the urine provide a direct measure with daily exposure of phenoxyethanol. The modeling that went into her development of the model used a wide range of measured data to develop the model and inform the parameters that needed to be included. That's included in this slide where we have an extensive *in vitro* and *in vivo* data sets following oral and dermal exposure in rats and humans were have concentration specific determined in plasma or urine samples and also *in vitro* skin conducted and all show you a presentation of our model predictions based on that data. The point of this is the administered dose range was very broad from .1 to nearly 500 the low dose exposure that we expected to occur for risk assessment of exposure from cosmetics and personal care products to the higher toxicity studies that were conducted did understand what the toxicity was.

The model structure shown in this slide the code was written in Berkeley Madonna and because we had two different routes of exposure we had a route of exposure for dermal and oral uptake that required the use of solubility limited absorption and that was based on observations that were made from the oral data that was generated for rats, and we were tracking the parent compound and because it's so readily converted we had to have a sub-model for the metabolism and the formation of the analyte to be able to track it and validate the performance of the models.

The dermal exposures included ability to acclude where we didn't have any evaporative loss or removal or unabsorbed chemical that would be applied on the skin and it also had the ability to wash and remove any of the unabsorbed compound [inaudible] applying and removing cosmetic ingredients. Metabolism for phenoxyethanol conversion formation of the [inaudible]

was described based on the Vmax and Km because we did see saturable metabolism in some tox studies where we needed to include in that description.

Here are a few slides, we have the plot on the left showing very good agreement between model predictions of phenoxyethanol and [inaudible] acid in plasma in a rat study following oral dose. And the plot on the right is the concentration time profile of radiolabeled material following oral dose of phenoxyethanol to rats in specific tissues fat, liver and kidney and we have a quantified levels of [inaudible] acid in urine as a function of time and we see correlation and good predictions between our models and the observed or measured data. For the dermal human exposure this is subset of the data set we had available.

We had one *in vitro* skin study conducted in two different formulations, so we headed to gel formulation that included 30-minute exposure period followed by a wash off procedure and then they monitored the penetration of the material into the receptor fluid for 24 hours and then body lotion application where the body lotion contained phenoxyethanol onto the skin and the permeation regulator material through the skin and in both of these formulations were studied at two different dose levels so that was helpful as well. The roof saw good correlation between the model measured values, and we have *in vivo* subjects that topical exposure to phenoxyethanol ointment and they monitored the [inaudible] in the urine is a function of time following a single exposure of subject a or repeated exposure for subject B, C and D. We had good correlation between her model prediction. You can see that the measured concentration for subject B were lower than model predictions. We don't know why that would occur. We were over predicting but it could be that the material was not applied effectively. Maybe there is a wash off or removal step either by the subject or off on clothing or something that the individual may have been wearing.

There is also an extensive data set that was available in 637 subjects where there was biomonitoring data to quantify the amount or concentration of the acid in adults and we use this data. We did not know what the dose was but we calculated it based on what we understand to be the urinary output for a given day and we assume it was a steady state [inaudible] back— from the parent compound understand what the dose level was so when we do that we can put that value into the model and run the simulation to get a prediction for the amount it should appear in the urine and that's what's plotted on this graph. Again, the correlation between the model predictions in the measured biomonitoring data in urine.

The next part of the assessment and this is again a bit of a review but evaluates the model performance or competence in our model prediction. This is based on criteria that was specified in WHO to 10 guideline and we looked at biological basis and we rated this is high because the model parameters and structure had reasonable biological basis and they were consistent with the available data in several experiments using a single set of input parameters for each species. We do not have to change anything in the model to run the simulation we saw good correlation between the predicting and [inaudible]. For model simulations we rated that high as well because the model reproduced consistently all the pharmacokinetic data including the shape of concentration time profile in the test species and with human data that we had the urinary output. Those [inaudible] showed good correlation. We also had the reliability assessment and the model simulation was rated high due to the comparisons between the dose metrics that were measured that were relevant to the bile [inaudible] species and exposure route relevant to the toxicity and risk assessment. We did do a sensitivity analysis and I did not include that in this today, but it is in the paper and available for anybody who's interested.

Here's a little bit about the dose metrics. A mentored that we measured the model include predictions for area under the curve and C_{max}—appropriate dose metric and that is based on the relationship are seen on left inside between external exposure and internal concentration that are predicted by the model and there are two studies one was an oral bullet study and the second study was oral drinking water study in our model simulation we see a trend between the [inaudible] low-dose oral and low-dose drinking water studies. Then increases in internal concentration when you increase the dose where they saw the low effect value for the oral bullet in the drinking water study. You do not see that for the [inaudible] correlation where we have a higher C_{max} relative to in the bolus relative to the drinking water so there's no trend. From this we concluded that the [inaudible] was the dose metric appropriate.

The refinement of TK uncertainty factors our ability to do that again was based on WHO criteria. We are using a PK model based on biologic based. The model parameters were also rated to be high because we had chemical specific data. We were using data specific for phenoxyethanol. It was applicable to the dose cost species and route and life stages relevant to the risk assessment. We also had for the dose metric we identified are hypothesized the [inaudible] toxicity following exposure to phenoxyethanol.

To this we were able to refine the inner species uncertainty factors based on our understanding of the ADME processes be a route and species for ratting humans. The enter species TK default factor was reduced to one. The talks code on iMac portion of the default inner species uncertainty factor of 2.5 was preserved and that was to preserve the inner species extrapolation which should be adequately protective and that leads to refinement of differences from 10 down to 2.5 and a total uncertainty factor from 100 down to 25. That is our uncertainty factors that we refine based on our model development and validation. With into the model and we needed to—we used to run [inaudible] following cosmetic and personal care product use and that was driven by the notes of guidance exposure data that was published by the FCC notes guidance for all the products you can see in the table and we assume that the phenoxyethanol and products the maximum concentration of 1% conservatism built into the model we also assume that the washout times were [inaudible] application frequency which again is conservative. For example, the shower gel when you take a shower you rinse it off before you step out and draw yourself. Typically, the if the dosing frequency is 24 hours then we set that wash off time for any of the material that would be remaining on the skin would either be removed or observed or stay the—are model predictions.

From this we can calculate an aggregate AUC and C_{max} based on the sum of these simulation runs for each product exposure and that data can be used to predict and calculate what the internal calculation would be following these exposures we also ran the model simulation for 13 week rat drinking water for the NOEL was [inaudible] again with the human aggregate defiant in the previous slide the curve values for both phenoxy [inaudible] and I mention those conservatism built into it also for the oral exposure hundred percent of ingested dose was absorbed found in toothpaste mouthwash and we also assumed that removable unabsorbed according to the application so from this we can then in this case it would be the area under the curve for the rat divided by the internal for the human in this is simulations and from that we can calculate what our margin of internal exposure would be in him showing for phenoxyethanol value of 78 because we refine our total uncertainty factor default from 100 to no appreciable risk following exposure to humans at a maximum concentration, so in summary for the traditional risk assessment applying the default uncertainty factor are of 100 would be that would require extrapolation the drinking water NOAEL to a reference value of and the use of 100 certainty factor however because there were certain—that was available was incorporated in the development of the model information was used to adjust the inner species

extrapolation and we were able to refine that using the to extrapolate human dermal exposure 15 milligrams per kilogram per day and here again a refined model internal exposure between the NOAEL and rats and humans would be 25. Calculated margin of internal exposure or aggregates in aggregate cosmetic use in adults refine total of 25 under normal use for phenoxyethanol at a concentration of up to 1%. Importantly the refine chemical specific uncertainty factor were derived that included some exaggerated product use. With that I have some references as well as contributors of this work

Clewell: Thank you, John. Are there any questions for John on his presentation? Maybe you could say a few words about how this is being submitted as am OECD case study?

John Troutman: Phenoxyethanol, as Harvey indicated, Cosmetics Europe, there is a number of case studies that they have taken on to set the strategy for long-range safety assessment. The idea was to get people from industry to think about how we can develop and apply safety evaluation of chemicals in the absence of animal data. In this case an example you can see there was an expensive data set that included a number of studies that used animals to identify the toxicity as well as the pharmacokinetics. But ultimately, more routinely were faced with of the challenge of developing these models and using them to make informed decisions on what the exposures are and what relative risk would be following the exposure and how do we go about doing that in the absence of data. Phenoxyethanol was a case study in one of those projects; we are actively working that now, developing PBPK and applying them with the mind of ignoring the available animal data and evaluating the potential risk of exposure for phenoxyethanol in consumer products.

Clewell: Thank you, John. The first three studies were caffeine, phenoxyethanol and parabens. I can't remember two or three parabens and they were done under different assumptions regarding what the available data was from no data at all to data for read-across to considerable data on the chemical of interest in order to try to propose approaches that could be used in the future given the regulatory situation in Europe were cosmetics cannot use ingredients or new ingredients tested in animals. How that might go forward and how OECD respond in terms of whether they feel it is a valid approach for the future. A very important step forward for toxicology, I would say.

Our final speaker is Shruti Kabadi from CFSAN, the next building over, I understand. She is a pharmacologist and she is a toxicological reviewer here at CFSAN and has a good deal of pharmacokinetic skills and she is going to provide us with some examples of route-to-route extrapolation here and I am looking forward to hearing that myself.

Examples of Route-to-Route Extrapolation Conducted at the FDA Center for Food Safety and Nutrition
Shruti V. Kabadi, US FDA, College Park, MD

Can everyone hear me? Thank you. Thank you, Dr. Clewell, for the introduction. I am delighted to be here at this colloquium. The title of my talk is Applying Route-to-Route Extrapolation for Food Ingredients. The speakers before me have done a great job of describing all the concepts on the route to route extrapolation, but what I would like to do in the next 30 minutes or so is to interpret and describe how those concepts apply to food ingredients or those could be used for evaluating food ingredients. I will be beginning a brief introduction and as I am describing the considerations and using case examples to show applications of route to route extrapolation, those are going to be explained in the context of food ingredients. Before I get

into my talk, I would like to say that I have nothing to disclose; all the views expressed are of my own have nothing to do with the views of the FDA or its policies.

Route to route extrapolation essentially means extrapolating internal dose from one or more routes and then predicting effects based on the internal exposure instead of six external exposure level. If you see the schematic food ingredient is the oral route and then there is non-oral route which can be in elation cut dermal and you have external dose on both sides and how you factor into the pharmacokinetic chemical to get to the internal dose and use that information to extrapolate between internal doses for predicting effects. The basic assumption of route to route extrapolation is data from studies based on alternate route of exposure appropriate for use for evaluating a chemical after exposure via the route of interest.

There are a lot of factors that affect route to route extrapolation and the first one being chemical characteristics of the chemical, which is its molecular size, molecular weight, partition coefficient, P KA, solubility, volatility, reactivity of the chemical, etc. The dosing or administration plays an important role as well as the information on the dosing rate, frequency of dosing, duration of dosing if it happened over a period of time and method of administration also need to be taken into consideration. Finally, the exposure related factor that play an important role in determining what effects we could be dealing with and those factors include contact site, contact duration, contact area, blood flow rate, diffusion barriers involved in the pharmacokinetics of the chemical, etc. One commonality between all of these factors is the pharmacokinetics of the chemical play an important role in this is something that the speakers before me have described in detail. It is important to take into consideration the [inaudible] hepatic metabolism as well as elimination of the chemical after exposure via all the routes of exposure that are under examination before attempting a route to route extrapolation.

This is a schematic that comes from a report that was just drafted after the workshops and South Carolina were all the experts got together to assess route to route extrapolation and provided recommendations. This schematic was also there in Dr. Clewell's presentation so to summarize this schematic presents evaluation adequacy of existing data to determine route to route extrapolation is a possibility or not. It is a decision tree, so it begins adequate toxicology data available for the route and at least one route and if the daily that are available then toxic effect because of the chemical exposure or contact itself [inaudible] portal of entry affects. Because a portal of entry, this is probably not a candidate for route to route extrapolation is the effect because of systemic exposure then it is a candidate for route to route extrapolation. If you look at the other arm, if you do not have adequate data on that particular chemical, you have other options available. One is utilizing [inaudible] relationship and trying to determine whether there is a structure and analogue of that particular chemical that would have relevant data available which could be extrapolated.

When you think of route to route extrapolation for food ingredients, there are certain things to keep in mind. First the safety assessment of food ingredients is performed primarily based on oral toxicity data. Dr. Troutman and Dr. Lipscomb in their presentations highlighted how extrapolation could be performed going from oral to non-oral. In my talk my focus is going to be going the other way how you use non-oral data for evaluating a chemical after oral exposure. Although the assessment of food ingredient has been on oral toxicity data sometimes [inaudible] non-oral studies may not be available or of adequate quality to evaluate food ingredients. In that particular case, route to route approach becomes a possibility is data from non-oral studies such as inhalation could be utilized for evaluating a food ingredient. Before we determine the route to route extrapolation can be performed or not, the first step is to evaluate the relevance available of predicted data from non-oral studies to determine whether that

particular food ingredient is indeed a candidate for route to route extrapolation. That involves two steps.

The first is to examine the pharmacokinetics of that particular chemical after exposure under examination and examine the overall toxicology. Or so will be talking about examining the PK or pharmacokinetic equivalence between the different exposure routes and that brings us to evaluating external data could be done qualitatively by assessing the available data and literature on distribution metabolism and elimination of that particular chemical or if more data are available than the assessment could be done quantitatively by estimation of pharmacokinetic parameters in the speakers before me have talked about some of them already but these could be [inaudible] bioavailability [inaudible] etc. Dear classical and physiologically based pharmacokinetic models that are available that serve as resourceful tools for examining pharmacokinetic equivalence between different exposure routes.

To provide you a background on different types of modeling compartment PK and non-compartment PK. Compartment PK models as the name suggests assumes that the body can be divided into two different compartments and the schematic here shows a two compartment model between the [inaudible] and in general the central come apartment—compartment different tissues do and then there are non-compartment PK models which have the opposite assumption that the body cannot be divided into two compartments and instead when you evaluate internal you evaluate the whole exposure in the system itself and you do that by mathematically calculating the area of the curve that you get after plotting the plasma or blood concentration so you can see in this schematic here. It is literally the area under the curve of that graph.

When you have relatively more amount of information regarding physiologic then you get an opportunity to [inaudible] this is been very well described in the talks before mine today but to refresh everyone's memory, the schematic here shows presentation of a simple PBPK model. They are built after making assumptions on the physiology of pharmacokinetic chemical based on the information available in literature or as a result of data obtained from [inaudible] studies that were performed to build the model and once the model is built is run and validated again based on the data that you generate from these studies or data that might be reported in the literature. Over time, as more and more information comes into the picture you get the opportunity of the refining the model and you revisit it and maintain it while you are trying to applied for evaluating the chemical under question. In short, PBPK modeling provides an opportunity to evaluate a chemical going from external dose to internal dose and that dose is taken into consideration as you predict effects of the chemical.

The second part of examining the relevance of availability data for route to route extrapolation is to determine the toxicological relevance of all of the available data. This involves asking some questions. The first one is whether the effects reported or observed are they due to contact exposure, portal of entry effects, or systemic exposure? The second question is are there differences in the type of severity of observed or are there differences in the severity of the effects? Then you evaluate these effects [inaudible] internal exposure because evaluated from pharmacokinetically prescribed in my previous slide and finally if you do have any information on the potential mechanisms of action it is important to evaluate potential mechanisms of action in the context of the data that are available from the different exposure routes to determine route to route extrapolation is a possibility.

I am going to present in front of you some case examples where we evaluated the adequacy available for pharmacokinetic data as well as toxicological information to determine whether

route to route extrapolation could be performed for food ingredients and if yes how can it be done. The first case example is styrene. Styrene is a chemical that has been looked at by several investigators over the years and there are PBPK models on styrene that have been reported in the literature. Regarding food ingredient, styrene-based polymers are regulated for indirect food contact uses. When we reviewed the available toxicological data on styrene, we identified oral inhalation and exposure studies on styrene that reported increased incidence of tumors in the in the lungs of rodents. The question that we asked after evaluating the relevant information was whether there was a need to utilize data from inhalation study to evaluate styrene after oral exposure and if yes then how could the internal dose be calculated? This could be further be utilized for determination a point of departure. Before I get into styrene, I would like to mention that the human relevance of reported carcinogenic incidence in rodents after styrene exposure subject of debate and that is beyond the scope of today's discussion. It is something to keep in mind.

First, we examined the pharmacokinetics of styrene after oral and inhalation exposure, and we performed some sort of semiquantitative assessment in the beginning, by surveying the available pharmacokinetic data in the literature on this chemical. And we basically considered the ADME of this chemical, which is summarized on this slide.

First, we found out that 70% absorption of styrene has been reported after inhalation exposure versus the 100 percent adoption after oral exposure. Styrene after oral as well as inhalation exposure is primarily metabolized by the cytochrome P450s mainly in the liver and also to some extent in the lungs. There are similarities in the biological half-life that of been reported for styrene after exposure in the range of 8 to 9 hours and some studies have reported an initial elimination phase with a half-life of 26 hours followed by a longer or slower elimination phase with the half-life of 13 hours. As I mentioned models of been published on inhalation exposure and they have included metabolize into several equations particularly the seven eight oxide.

We also evaluated the available carcinogenicity data on styrene after studies in these data are from two studies that have been reported after inhalation exposure to styrene. Studies were performed in different strengths. It also shows the exposure levels that were tested in the inhalation studies. The exposure study was for shorter duration conducted for seven weeks where the assay was conducted for two years and in both studies carcinogenic incidences were reported in the lungs. These were reported at lower levels after inhalation exposure [inaudible] after exposure. I would like to mention that when we reviewed the studies, we identified deficiencies with the carcinogenicity study performed on styrene which seemed to be less reliable than the data reported after inhalation exposure to styrene.

We then went back to evaluating the data sets that were in front of us and we attempted to reconstruct a simple PBPK model shown in the schematic this was based on single inhalation exposure to styrene [inaudible] we used simple we focused on the chemical itself but did not look into the metabolite because the purpose of the modeling was to compare directly the levels of styrene in the blood as well as tissues in inhalation and oral exposure. The data was validated based on the information reported in the literature and as I mentioned we modeled the changes in levels of styrene and blood in some tissues as a result of the PBPK modeling. All the metabolism in this model was lumped under liver itself because although metabolism of styrene in the lung has been reported the liver is the significant contribution to the metabolism of styrene. As I mentioned metabolites were not examined.

This slide shows a direct comparison between oral and inhalation exposure of styrene in the redline curve basically represents changes in levels of styrene in the blood. The blue line is a behavior in the liver and the green represents what happens to styrene and fat in the black curve is the data reported in the literature. Qualitatively examining these curves, it appeared that there are no marked differences in the decline of styrene and blood and tissue between the two exposure levels.

We also validated these data different exposure levels in this table summarizes the validation at the exposure levels. We tried to increase the exposure and see what predictions of PBPK model was giving us and we compared these predicted and those that have been reported in the literature and we compared the two. The model was giving us a fair validation for utilization. Now these ratios were relatively higher for the inhalation exposure level than the oral.

Just to summarize the conclusion from the PBPK modeling work we observed the inhalation exposure after oral as well as inhalation the internal exposure as well as inhalation exposure to styrene [inaudible] and in both cases we observed the metabolism showed saturation at higher external exposure levels. In both cases styrene partitioned into fat more than the other tissues in the concentration of styrene and blood and all evaluated tissues declined within 24 hours. As a result of the firm a kinetic evaluation as well as the examination of the toxicological data we concluded inhalation data could be used for evaluating styrene after oral exposure, styrene is a candidate for attempting extrapolation using inhalation data.

The next question is how would you do that? As we know inhalation exposure or the external inhalation exposure and studies reported in terms of PPM in error. Oral exposure the daily dose is expressed in terms of milligrams per kilogram per day, so we utilize the principles of inhalation and included physiological parameters into the equation to calculate it into internal dose expressed in terms of milligram per milligram per body weight per day. This is based on the principles of inhalation dosing which have been utilized by EPA for years, but we tried to calculate species with physiological factors and included bioavailability into the equation for external and spoke sure which could be [inaudible].

So just to break it down. We have the exposure in parts per million. We first calculated it in terms of milligrams per milliliter and then we factored into the equation the time of exposure in inhalation studies the duration of administration generally a six hours five days a week so we factored that into the equation to calculate the time of exposure and from there we calculated a daily dose by including the ventilation rate and then the next step was to include the pharmacokinetics of the chemical itself that is a bio availability calculated based on the model and that is included in this equation and you end up with internal exposure expressed in terms of milligrams or kilogram per body weight per day.

We utilize the conversion for converting the inhalation exposure reported in the inhalation gentleness city ranging from 20 to 160 ppm into the equivalent internal dose or internal exposure in that range from 17.7821 42.25 milligrams per kilogram per body weight per day which could be taken into consideration as a date is evaluated for estimation of point of departure.

There are some considerations to be kept in mind when such route-to-route comparisons are attempted based on inhalation data in the first one is this conversion protocol is useful for calculating equivalent internal dose but only for compounds that are volatile and by that I mean substances that have a high vapor pressure that would be absorbed into the system of

calculation instead of getting accumulated into the lungs. That brings me to the next point that this cannot be used for substances that may not be fully vaporized upon inhalation exposure. This cannot be applied for substances that demonstrate the wash and washout effect upon inhalation exposure and that means sometimes you have chemicals that the inhalation occurred most of the, goal is out and that is the wash in and washout effect which, for those chemicals you can't use this protocol. As I showed in the conversion itself appropriate physiological values are required to perform this conversion and in this particular case for specific values for alveolar ventilation rate.

Now styrene case example represents an examination that we were successful in attempting in route-to-route extrapolation and that was the data on adequate for use for this conversion. However, that is not always the case. Now for the next 10 minutes I will describe case examples where this did not work. The first one being [inaudible] now we use the same approach we evaluated the available pharmacokinetic and toxicological data and when we looked at the pharmacokinetics of some such as fluoride, oxide and some containing substances such as [iaudible] and the metal itself we observed that the location and mechanical appearance of the particles from the lungs really affected its clearance from the lungs itself and led to increased retention rate in the long which is not affected when you consider or examine the exposure to cobalt and it's interesting the particle size of cobalt salt played a very important role in influencing the long retention times or clearance from the lungs itself.

The conclusion from the PK examination was the profile of cobalt salt were different based on the physical chemical parameters. We examined particle side, but studies have reported ionic charge vulnerability and other back errors that could play an important role. When we used from a kinetic modeling, we were able to calculate bio availability which were lower approximately two percent although we were not able to calculate bioavailability of inhalation exposure. Looking at what was available in the literature we were able to determine the systemic after inhalation exposure to cobalt metals is low and that's partly because a lot of the chemical itself is retained in the lungs and is slowly released from the lungs overtime. As shown in the table in the slide before we directly compared the profiles of cobalt oxide between inhalation exposure and oral and determined those were dramatically different. All the factors such as six which may play an important role on the profile of cobalt salt they were not taken into consideration as the data were not available in the literature including those factors into our examination. There is one thing I would like to mention on the toxicology of the cobalt salt and that's that a lot of the incidences reported after inhalation exposure to cobalt salt particularly [inaudible] have been reported to be a consequence of the lung inflammation which results from the contact exposure. So, there is an indirect entry affect [inaudible] which may play a role in the conclusion based on the inflammation we concluded that inhalation data cannot be used for evaluating cobalt salt after oral exposure.

The next case study was on try the melamine and we evaluated the data after exposure I would like to mention that after evaluating the data, we identified some severe deficiency with the design which reduced our confidence in the results of the oral exposure studies. We also evaluated inhalation data. When we looked at the pharmacokinetic oath of this chemical between, we didn't look at inhalation but looked at dermal studies, we evaluated the pharmacokinetics of the chemical between oral and dermal exposure and we observed that although both the chemicals after oral and dermal exposure gets rapidly absorbed there is a slow internalization of the dose after dermal exposure which leads to a significantly higher systemic exposure after dermal exposure to Triethanolamine. This is proportionate to approximately 1600-fold increase in AUC. We also observed severity of the expected toxic

outcome that would result from these exposures after oral and dermal exposure to Triethanolamine. There is one aspect of the examination which is on the slide and that is we also looked at structure analog of Triethanolamine one being [inaudible] which there is a lot of data available. There are some similarities in mode of action, the main one being most of these chemicals have been reported to elevate the synthesis and up take of choline in the hepatic cells which contribute to the hepatic incidences however the internal exposure of Triethanolamine that would be required to be correlated with this effect is significantly higher and that makes the data from dye for melamine not very relevant for the investigation of Triethanolamine peer keeping in mind the oral and dermal data available on Triethanolamine. We concluded if we extrapolated and correlated the dose based on dermal data to evaluate Triethanolamine after all exposure that would be an estimation of the toxic potential and therefore we did not attempt this extrapolation.

Just to summarize I would like to emphasize that the safety evaluation of food ingredients primarily rely on the available order data however when the available date is not of adequate quality our date is not available then there is a possibility to attempt route-to-route extrapolation based on the non-order route. For that it's important to examine the pharmacokinetic chemical between the two exposure routes and then to determine the toxicological relevance from data of the studies for evaluating the chemical after oral exposure. When you do have that option and if you could do that based on inhalation data then an equivalent internal dose expressed in terms of milligram per kilogram of body weight could be calculated on incorporation of species and ventilation rates and factoring into the bioavailability of the chemical and this value could be used for estimating a point of departure.

There are some challenges in the way of route-to-route extrapolation for an assessment. The speaker before me has explained this and to refresh everyone's memory the first challenges portal of entry affects. We saw some of that in the cobalt examination which is why we did not utilize data from inhalation studies for evaluating cobalt salt after all exposure. Modes of action which need to be considered and often times that information may not be available and sometimes when it is available it would be route to route extrapolation. There are several factors such as age which may introduce variability in the PK itself. It's evident to some extent from the talks today there are some inconsistencies in the route-to-route methodology used by different organizations and that is why things we have today are helpful because you can exchange your ideas to see signs of commonality. I would like to emphasize that there are a lot of differences in these examinations which I tried to showcase using the case examples today and this evaluation is done on a case-by-case basis, it sometimes works and sometimes does not which is why there are no generalized steps that could be followed. Obviously, the pharmacokinetics and the toxicology have to be evaluated but this examination for four ingredients is performed on a case-by-case basis.

I would like to leave everyone with the references that went into my presentation and I would like to thank my FDA collaborators particularly Dr. Jeffrey Fisher, who's on the webcast and Dr. Jason Aungst, who's in the room. I had the opportunity of collaborating with Dr. Patra Volarath and Dr. Janet Zang on some aspects of the work. Dr. Mattia and Dr. Roth are retired but they contributed to some parts of the work. Dr. Nikki Smith was an ORISE fellow with us who contributed to the styrene project and Benjamin Hung contributed to the cobalt work. With that I want to thank you all for your time and attention and if you have any questions, I would be happy to take those.

Clewell: Any questions? Related to the talk?

Roundtable Discussion
Moderator: Harvey Clewell
All speakers
Jeffrey Fisher

Harvey Clewell: I think it is time for us to form the panel, if the speakers would come up, you can move to the table, and the Johns can join you. I will stand, thank you. At this point I would like to take the opportunity to introduce my Co-Chair who is in Arkansas at the National Center for Toxicological Research, Dr. Jeff Fisher. Jeff are you online?

Jeffrey Fisher: Yes, can you hear me?

Clewell: Yes, we can. Very good. For those of you who don't know him, Dr. Fisher has been doing pharmacokinetics probably 40 years or 50 years or so. He and I met at the Wright-Patterson Air Force Base, we were both in the Air Force, military, and he actually was working in both the laboratory and doing PBPK modeling. I was just a modeler. Then he went to Georgia where he was chair of the Department of Environmental Health and then to NCTR where he is now senior scientist and getting ready to kick back, right? All my friends are retiring, except for me. At any rate, thank you for joining us, Jeff, and I asked Jeff to come up with a few questions to get to discussion, so Jeff, would you like to pick one of those and see if we get our panel talking.

Fisher: Yes, before I ask a question Jason promised we could go to Hilton Head for this meeting and I don't know what happened.

Clewell: Promises, promises. That's where the route-to-route meeting was. That was at Hilton Head. First and last time EPA pulled out off.

Fisher: It was a great meeting and this meeting is also a very good meeting. I am listening to all the speakers. I have a fundamental question and I am not sure if anyone has talked about it specifically. As you go to lack of data and you are studying the used cells, have the speakers been involved or thought through procedures to just use cell data for route-to-route?

Clewell: Actually, I forgot to check. Alicia are you un-muted?

Alicia Paini: Hello, can you hear me?

Clewell: Very good. Actually I would like to pass out to Alicia to talk about because I think the guidelines that you are developing trying to keep in mind the shift of modeling from use of *in vitro* data could you comment on what's going on with regard to determining the kind of studies *in vitro* studies that need to be done?

Paini: I mean, if we look at the *in vitro* parameterization of the models when we talk about route-to-route extrapolation [inaudible] we see there are some elements that can be measured *in vitro*, and these can be input into the model. We know if we want to link the PK model as we have seen in several slides during the presentation to more mechanistic [inaudible] we need to data for instance, we need to take into account the information when you have *in vitro* data measured can be some cofounders that can influence your prediction, your *in vitro* measurement, let's say. Then we touch upon the free fraction of chemicals that is *in vitro* system because we know *in vitro* system are different from *in vivo*, so they have different

setups, so we need to take this into account. The guidance document in that sense touches upon it but does not give any specific information on how to do it but says it's a possibility. It should be taken into account in the measurement of *in vitro* data. This is from my side, and I can say until now, so I don't know if I understood the question correctly.

Clewell: Yes, I think that was an answer to the question. I was wondering some of your colleagues there at ECVAM I think were working on developing good *in vitro* practice?

Paini: This is one important issue exactly, if you look at the OECD we don't really have test guidelines that actually cover the kinetic area and so we actually have to use a guidance document on good *in vitro* modeling practice in order to at least have reliable data to be used in the parameterization. There is a lot of ongoing work and we are also trying to actually look into the test guidelines really how can they be applied. First of all, there are some for *in vivo* but for *in vitro* there is nothing so from a regulatory context how we can use the data and it is actually something we are currently discussing this week on how to push forward at the OECD for the kinetics version. For now what I can say is use this guidance document [inaudible] was the leading person of this huge effort and it is already published in the OECD series so it shows the principles of how you can use good *in vitro* practice and it can be used for parameterization of PBK models.

Clewell: I know for my part I can say there is been a huge amount of activity in the last 10 years and it's amazing how much the state-of-the-art has in prove for *in vitro* metabolism measurements over the last 20 years. People like Brian Lake and [inaudible] in Europe have really determined what are the characteristics of a good study and how what one can get a wrong number by not considering different factors. [Inaudible] the people who have developed this at the University of Sheffield have been very studious in documenting how they do things, what needs to be considered so there is a really strong state-of-the-art for the right way to collect *in vitro* metabolism data, so that was the real issue just 15 years ago but now I think there is no excuse for saying oh well the *in vitro* metabolism is so uncertain we don't know the right way to do it. We do know the way to do it and whether it's microsomes or hepatocytes, you can get the same answer, except in cases where you need the intact hepatocyte for multiple metabolism steps, so you know when you have to use what. That part has reached maturity, I think, and we know how to use it in the models. So we are at a point where I think the next big question is going to be when there is been reluctance by regulatory agencies for using the PBPK models that have a lot of animal data showing that they worked, how it is going to be when we have a PBPK model where rely on *in vitro* data alone and that's what I mentioned the guidelines that Alicia is working on are the first one to actually try to grapple with the question of how do you include the future of risk assessment when you are looking at that issue of getting regulatory acceptance. Any comments from her other speakers?

John Troutman: I had a comment to add. I think really the application of an *in vitro* tox data could rely on the development and use of the PBPK model for understanding the concentrations within a given tissue that may be appropriate for testing within that cell-based model and to think about whether or not metabolism would be occurring or be involved in the activation or the detoxification mechanism. The cell-based models are typically it doesn't have a lot of high metabolic activity unless it's for the liver and these are still discrete models where they have a specific concentration incubated at a specific time point we need to think about how we can implement more physiology or more of the dynamic changes we know that occur *in vivo* that may be *in vivo* but if we can extrapolate that hazard is and extrapolate that hazard from the *in vitro* model relative to the tissue concentration using PBPK it will help inform what the next decision needs to be made for the risk assessment. For me I think about that is rapid

decision-making where we generate data and think about what the data suggesting and apply that.

Clewell: That's a good point. I have been very impressed by the work that Rusty Thomas has directed at NCCT along these lines of rapid decision-making application of metabolism and modeling. John.

John Lipscomb: In 2014 the US EPA published some fairly importance guidance in my perspective called data-derived extrapolation factors. It was a [inaudible] guidance that was already available [inaudible]. This guidance and the authors of the guidance took careful pains to address the proper use of *in vitro* data and health risk assessment and did so from the standpoint of both pharmacokinetics and pharmacodynamics. Two things they stressed relative to dose-response data is observations made *in vitro* needed to be made in tissues that were relevant to the target tissue *in vivo* as well as a concentration producing effects *in vitro* needed to be compared to the concentrations that might be attained *in vivo*. The second point was that the data could be used quantitatively to address differences between species from animals to humans as well as among the human population in that the quantitative basis for the comparison was at the level of the concentrations that produce the response the points of departure in for example animals and human so that's an important distinction that can be made between EPA data-derived extrapolation factors in IPCS chemical-specific adjustment factors specifically on the use of data, importantly the high-throughput data coming out of the ToxCast at the time.

Shruti V. Kabadi: One thing I would like to say I am in agreement, this whole aspect of improving future study design and I think *in vitro* studies are the best and fastest way to look at it, validating what we have and defining going forward. One aspect which is fascinating to me which I have seen that has exploded is examining kidney and renal transport especially when it comes to assessment of a lot of chemicals where elimination is a rate-limiting factor. *In vitro* studies provide an avenue for examining mechanisms such as renal reabsorption, secretion which is something that would be very difficult to model 20 or 30 years ago, when this was in the beginning stages.

Clewell: Yes, that is true. I feel like transporter research is about the place that metabolism research was in the 1980s. They're getting a handle on it and putting names on it but no reliable methods for getting numbers, Vmax and Km, for the transporters. But that will come just like it did with metabolism. Eventually will have panels that will be able to do it with transporters and it will be possible to feed that into the models as well.

Jason Aungst: Online we have some related questions. It goes toward an examination of TCE. Is metabolism outside of the liver considered? We talked about metabolism *in vitro* of the liver but how is metabolism in the lung and other extrahepatic metabolisms considered in these models?

Lipscomb: Before I punt and send it to Dr. Clewell, I will tell you Hugh Barton and colleagues did a study on extrahepatic metabolism of TCE and I think it was Mel Andersen that said the best answer always is it depends. It depends upon the analysis for which it was conducted. It is been considered most physiologically based pharmacokinetic models place the site of metabolism in the liver, that's a default position to be refined as conditions warrant. Harvey, what would you like to add?

Clewell: If you are worried about while-body clearance, then the liver is king so it is generally presumed safe to ignore extrahepatic metabolism, but if you're worried about toxicity in the lung, kidney or skin then you better consider metabolism. It is been modeled with isopropanol and [inaudible], so it is not something that hasn't been done but it's only done—who was it that used those precious words “fit for purpose”? It was you, right?

Lipscomb: It was not me; the first person I recall having set fit for purpose was our colleague Betty [inaudible].

Clewell: Bless her heart, anyway you will find in the WHO guidelines expressed that the model needs to be evaluated for its fit for purpose for the intended purpose of application because the right model depends on what you're trying to do. That includes the time frame if you're worried about acute exposure, chronic exposure, route of exposure, what kind of toxicity. So, if you are worried about trichloroethylene lung tumors, you want to predict the production of chloral in the lung by metabolism in the lung and the clearance of chloral and that was the hard part. I was never happy we could do a good job of describing that, so I suggested you shouldn't use a model for the animal to human for the lung. For the liver, it’s dichloroacetic acid and trichloroacetic acid, and we have a lot of great data from a number of people that allow us to confidently predict that. For the kidney, again, it's a little more uncertain, the *in vitro* study to establish the metabolism parameters disagree by orders of magnitude. That's what determines can you use the model for this and then if you can, should you? It depends on what the mode of action is and what you are trying to model.

For skin as a good example, the dermal absorption is often associated with a good deal of metabolism that is seldom important for whole-body clearance and is important for delivery through the skin and how much of the metabolite is generated in the skin, so is it important? It depends. It certainly is doable; it requires the ability, the challenges of *in vitro* study measuring lung metabolism is tough because it's restricted to club cells and there is not that many on a population level it's a small fraction of the lung population. Most studies that have been done just chop up the lung and measure the whole metabolism, but it is actually isolated in the bronchial region which can make a difference depending on where the chemical is deposited or absorbed so it can get complicated.

With styrene there are nasal effects that are really irritation and then there are lung affects which are due to metabolism in the club cells. Also circulating styrene oxide from the liver; there is so much metabolism there that the metabolite comes back to the lung but at low concentration most of the tissue exposure in the lung is due to lung metabolism, so it is very complicated, and it depends on what you're trying to do. The good news is for most route to route extrapolation, what you want to do is compare the exposure that gives you the same blood level the internal margin of exposure or margin of internal exposure in European terms. When you do that it falls out by itself. You run the oral study; nothing matters except for the liver clearance. If you have a dermal study, you need to consider whether there is a likelihood the chemical will be metabolized in the skin. And lung you can always ignore pre-systemic clearance in terms of effect on whole-body clearance. If you just need a blood level for route-to-route it's not a problem. Shruti?

Kabadi: I would like to say hepatic versus extrahepatic also depends on what is available, so it's easier to find parameters for liver than for the extrahepatic metabolism which is what I've seen in my experience dealing with food ingredients. Sometimes parameters are available, like for styrene that are metabolic parameters for lung and liver but then the difference is so

significant that you have to make the decision. If I lump everything in the liver, will I account for most of it or not?

Clewell: Right, and the liver blood flow is 25 percent so inhalation, the liver then does not have as much of a clearance capability as it does for oral but that's accommodated in the PBPK model. You can actually do sensitivity analysis by adding a relatively significant level of metabolism and seeing what difference it makes and I've generally found it's not much. You can actually use a model to try to evaluate the uncertainty in your route-to-route extrapolation.

Aungst: I'm going to combine a few questions here that are related. So, these go toward use of PBPK models. For example, can the model be built for classic compounds and related question can these models be used for looking at mixtures, for example, selenium, lead, arsenic, caffeine and other compounds?

Clewell: Okay, that's a resounding yes and no. Actually, the case study I mentioned that they are working on now for the parabens is something where they are developing a model for the parabens class of chemicals [inaudible], so then it's just a matter of plugging in the chemical specific metabolism parameters and estimating the partition coefficients with QSAR based on structural difference. So anyway that part, you can model the mixtures, almost all interactions are competitive inhibition that is well-known it just means you have to duplicate your model for each of them and put a link between them with the KI in the liver, but we have done that in the past. It is straightforward; it's just a lot of trouble. So, you can then, [inaudible] has been involved in work in Canada looking at [inaudible], so yes absolutely, mixtures and classes of compounds. It's a right way to read across quantitative read across where you can actually determine what the impact of the different half-lives of these chemicals by doing an *in vitro* measurement of metabolism and for the unknown chemical and comparing it to some where you better data which is what they are doing in the case study.

You lost me with the metals. Yes, you can model the interaction between metals and it's harder than modeling metals in the first place. Metal models tend to be more empirical than chemical models because organic chemicals are pretty simple in terms of their transport and distribution. Thermodynamic equilibration, chemical activity as a driver. With metals, they are generally moved by transporters or they ride on ligands, they like methylmercury forms methylmercury cysteine, which is what goes around the tissues and it is transported into the brain and mistaken for an essential amino acid. So, you have to have abundant data on a metal to do a metal model, and if you wanted to do one that had interaction between two metals, you need abundant data on the interaction like with methylmercury there is an essential element, what is in the Western US too much and it makes the bird beaks curve, yes selenium, thank you. Anyway, there is actually laboratory work looking at interactions between methylmercury and selenium, so you have to do studies with the mixture.

Aungst: Another question. I think this is addressed by Dr. Lipscomb talking about looking at the quality and use of *in vitro* data, but this goes back to what was originally used in *in vivo* data. When making relations between route-to-route exposure in different studies, what is a level of confidence in making conclusions and models from older studies as well as newer studies which may have some uncertainty between those? And how do we go about increasing our confidence in studies we choose to use?

Lipscomb: So, the question is how do we increase our level of confidence in older studies?

Clewell: Yes, those you decide to use.

Lipscomb: Historically speaking, we need to go back to any study and see what was the data set available to the authors of the study at the time they made their observations and drew their conclusions. Many of the things that are written in the older literature like the stuff we wrote is the conclusions are completely appropriate at the time, but they obviously have no way to extend themselves given the advances in knowledge. The names of enzymes have changed but the fundamental biochemistry of enzyme kinetics is certainly not changed and neither has anatomy and physiology so sometimes we have to go back and do some deciphering. Additionally, there are on occasions errors in translation from studies published in German or Russian into English and those can be complicating factors also. It's all a great big puzzle in terms of putting the stuff together and the puzzle pieces we need to give the most weight to are the ones we really understand. We know the shape of the puzzle piece, the color, we know the box it came out of and when we know those things we can begin to put together a picture that is a little more readily discernible than if we go and pick up a bunch of stuff we don't know how it fits together. If we don't have, the bottom line for me however for me, if we don't have confidence in the data than we can probably at best use it qualitatively and if we are really careful, we can put it into the matrix in terms of certainty or quality. Cellular physiology has not changed in eons, and we need to put that hat on when we evaluate some of the older studies. I would ask you, Jason, is that enough of an answer?

Clewell: I would like to see if Alicia or Jeff have anything to comment on that? Maybe not.

Fisher: Hang on. I think that is a tough question and it can be specific to what you are looking at, old literature plus more recent literature. I don't think there is one easy answer and you have to be aware of how things were done in the day versus now more recently. That has been my experience and sometimes I have thrown out old data because it is old, but it is specific to summaries and that probably doesn't reflect the use of today.

Clewell: Yes, I have to say my own personal experience being a modeler is we are the ones unearth problems with published experimental data and then we get in trouble for not matching the data. But I remember one time we could not match data on styrene from Dow Chemical, I called and asked how they collected the blood. They said, we decapitated the animal and poured the blood through a funnel. This is styrene so no wonder we could not match the kinetics. We had to back estimate the concentration inhaled, but anyway, so there are some things done in the old days and probably still today. Actually, I find a lot of people nowadays doing studies who are not familiar with inhalation [inaudible' and the problems of doing experiments [inaudible] particularly metabolism experiments and they don't even understand you have to have a sealed system. It is a great fear of mine believing data that is not true. In an abundance of counselors, there is wisdom. In an abundance of experimental data, there is confidence. When you get eight studies that agree in a ninth that does not, I would not worry about the ninth study. So, that's kind of what you have to do to get a consensus data approach.

Aungst: Another question online. How do these models take into account when you use, when metabolism changes with exposure or dose and when metabolism is overwhelmed? How is that considered in models?

Lipscomb: There is a concept called kinetic maximally tolerated dose that I've been aware of since the late 1990s. It came out of colleagues in central Michigan. It relates principally in the context of risk assessment to thou shall not without very careful cautious deliberation extrapolate points of departure in the saturating range to human exposures. Now the way you

can understand what is a kinetically maximally tolerated dose or saturating dose is to run a pharmacologic model or collect pharmacokinetic data.

The mode of action, there is a human relevance mode of action framework published by IPCS in 2006 or 2008. The framework is simple. When we see a mode of action that occurs at concentrations that are not likely to occur in the human or the concentration response relationship indicates this mode of action, here metabolisms, or the saturation of metabolism, is not likely to occur under the condition of human exposure, then findings generated under those conditions in the animals are under suspicion and perhaps not likely to be relevant to the human and I would add without further interpretation. So, saturation is important. We often see saturation in animal studies but seldom in the human, but we see saturation on occasion.

The difference between saturation *in vitro* and *in vivo* is the difference between night and day and that is because *in vitro* we have saturating concentrations of substrate, we have saturating concentrations of cofactors and the enzyme is free to run as fast as it possibly can, whereas *in vivo* we will see the constraints of delivery to the liver by blood flow and partitioning as well as the possible constraints of a non-replenishing cofactor system or cofactor system that cannot replenish itself as fast as the enzyme can metabolize a substance. *In vivo* you will have two additional constraints on a metabolic rate that you won't have *in vitro*: delivery of the substrate and regeneration of the cofactors. For those reasons we have to be very careful about determining the maximally pharmacokinetically tolerated dose.

Clewell: Kinetic maximal dose, okay.

Aungst: Another question: a lot of previous models revalidated by total radioactivity—do you think we can use those models for route-to-route extrapolation especially if parent metabolites were in radioactivity? I think you had mentioned something about that earlier.

Clewell: Handle with care. It's a huge complication and I've had trouble with models where the only data we had available was total radioactivity. You need some data to differentiate parent metabolite under those conditions and I think you had a lot of radioactivity data, John, but also had a few studies that allowed you to anchor things. Could you comment on that?

Troutman: The case for phenoxyethanol, there were PK studies that were generated, some data that was generated measuring both the parent compound in the acid metabolite and there were also studies that used radiolabeled phenoxyethanol and tracked just the radioactivity over time, and so both those data sets were important for helping us understand the kinetics as well as the *in vitro* metabolism data to show that when you convert the parent compound, nearly all of that material is used to form the acid metabolite. If you show from the *in vitro* data set or *in vivo* data that the concentrations of the metabolite was low relative to high metabolism of the parent compound, that suggests you have potentially many more metabolites that would be formed and therefore the total radioactivity you are tracking in the studies could be due to the sum total of all those different metabolites in different concentration time profiles in which those are formed, they appear and also how they are eliminated. It's a complicating factor and it is not, I think it's something that needs to be considered based on the available data and also potentially to drive more *in vitro* studies to better characterize that metabolism of the parent and what is formed *in vivo* in terms of is it a single metabolite or are there multiple metabolites?

Aungst: All right one more question online. What is the impact of formulation [inaudible] and how is that considered or managed between PBPK models when trying to do route-to-route

extrapolation? I'm assuming they are saying maybe your oral study has one vehicle and your inhalation study uses a different vehicle. Do you see differences in the parameters that come from that?

Clewell: The good point. The problem is well inhalation of vapors is always an error so that one's easy but dermal of formulation makes a big difference. The lipophilicity of the formulation for lipophilic chemicals just the fact whether it's occluded or not there's a lot that can cause variation in dermal uptake. For oral there's a big difference between aqueous and oil vehicles, and the kinetics is hugely different. Typically, much slower uptake with oil but it can be reduced or increased. Also an oil vehicle it will stress the liver and make it more susceptible to damage but we looked at pyrethroids with aqueous versus corn oil vehicles and had to introduce additional compartment, the corn oil vehicle itself was something the chemical could diffuse out of in order for uptake so it's almost like a pharmaceutical pill type model where you have the traveling dose that you are trying to get uptake from. I highly recommend aqueous in oral but when we did vinyl chloride we compared the carcinogenicity estimates using rats and mouse oral and inhalation and we got the same amounts for everything except corn oil. Corn oil, the potency was about eightfold higher and [inaudible] who is an expert on corn oil gavage said he had seen that a with the large amount of chemicals, that corn oil gavage does exacerbate liver injuries, so it is not just the changes in uptake; it also conditioning the tissue to make it more sensitive.

Kabadi: When you evaluate the toxicological data to determine whether it could be used for route-to-route extrapolation and the vehicle, as Dr. Clewell said, that is used to formulate that substance makes a huge difference. It's very important to consider that. In the styrene case example that I showed, for oral, the styrene test material had been prepared in corn oil. And then, when I started looking into corn oil, I came across some literature that Dr. Clewell just described that showed that corn oil really interferes with the pharmacokinetics of the system of the substance itself and that is why a lot of the effects you are seeing, although they are because of the chemical, they may not be at the dose at which you would be seeing them in a living system. If you look at these earlier studies done for volatile compounds, most of them are in oil. And fortunately, now there is data coming out that have come out showing comparisons between substances made with oil and substances other vehicles. You have the opportunity to consider whether a study could be used for toxicological evaluation or not. I think the same goes for inhalation exposure. A lot of the inhalation studies are whole body and now new studies are reporting [inaudible] is the way to go. A lot of the studies have been performed and been repeatedly used that are based on whole body exposure. I think it is the totality of information and overall quality of the study that needs to be considered, and vehicle is just a part of it.

Aungst: Any more questions from Jeff Fisher?

Fisher: Thank you, Jason. I have a question for the panel concerning uncertainty factors or on the risk assessment side, do you think there needs to be some adjustment if you use a route-to-route extrapolation and there is really no data for that route, would you use an uncertainty factor or not, or how do you deal with that?

Lipscomb: Hi, Jeff. There would be a fall back, and there would be a couple options there. If we are using a physiologically based pharmacokinetic model, then there are certain guidelines for model evaluation and model application that should be followed to determine what and how much limitation that no data or limited data impose upon the confidence placed in the model. On the other hand, if we have enough data to develop a model, then might we also have

enough data to interpret the dose response from the other route? If we have the data, we don't need the model, but if we don't have any data at all, can we develop and place confidence in the model? That's a huge gray area. In terms of putting another uncertainty factor on there in the context of NAS's original 100-fold safety factor based on [inaudible] ADI and safety factor, I don't think we need an uncertainty factor like that, but I think it would be a pass fail on whether we have enough confidence on the model from the second route where data may be limited for route-to-route extrapolation to be used in confidence, but I'm not a regulator.

Fisher: Thank you.

Paini: This is Alicia. I think also it would be relevant to take into account that you can generate for this type of information using *in silico* methods. If you don't have for one route for that chemical information, you can try to generate them using other approaches and then you can otherwise try to see if you can use a read across approach to inform that type of route if the chemical has an analog that has the same mode of action. So, something I think that John just mentioned is whether or not to go to uncertainty factors, but maybe it is better to provide more tangible information and more evidence and model them. This is my point of view. So, going down this road more than giving a new uncertainty factor.

Aungst: Thank you. Any more questions, Jeff?

Fisher: I don't think so. Thank you.

Clewell: Well, it may be that we are ready to move on unless you have anything else.

Aungst: Any more questions online? I don't see anything yet. Maybe we can move on.

Clewell: I have a couple slides I have to go through. Correct? Do you think it is in the same, oh, topics for future 2019-2020 colloquia. April: "Artificial Intelligence in Food and Cosmetic Safety." So, that is like the U.S. Senate. And then, that should be really interesting, though, it's forward thinking. And then May is "Integrated Approaches to Testing and Assessment—the Future of Predictive Toxicology." That is a good one. I really like the direction that ECVAM in particular is trying to move with the IATA, and I think that is essential to being able to use *in vitro* direct in risk assessment, having multiple assays and figuring out how to put them together.

So, apparently there are recordings. Would someone access that by going to the colloquium page? Also the slides and captioning, very nice. SOT at no charge, now, that's a deal when SOT gives you something for free, I didn't say that. Thank you for your participation. We appreciate your input, and someone's going to send you a link and we appreciate you completing that survey. Thank you.