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Safety Assessment Approaches in Young Children

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

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Children Matter: Using a Lifecourse Approach to Understanding Safety Assessment Needs for Children, Elaine Faustman, University of Washington, Seattle, WA

Early Life Development of Pharmacokinetic Pathways: Framework and Case Examples with Implications for Safety Assessment, Gary Ginsberg, Connecticut Department of Public Health, Hartford, CT

Break

Ensuring Safety for Early Life Exposures: Adequacy of Current Methods and Opportunities to Advance the Science, Susan Felter, Procter & Gamble Company, Mason, OH

Toxicology Challenges in Lifestage-Specific Safety Assessments, April Neal-Kluever, US FDA, College Park, MD

Roundtable Discussion, Peter Goering, Moderator, and all speakers
Welcome. Thank you for coming on this beautiful Friday morning to the eighth in a series of SOT FDA Colloquium on emerging toxicological issues. We call it “Challenges in Food and Ingredient Safety,” but really, many of the topics that we cover have been broader and have interest to all toxicologists at FDA and at other federal agencies and other SOT members. This is part of a MOU that we have with SOT to conduct four half-day sessions a year. This is the second year we’ve added. The program is chair by Dr. Allen Rudman from our Office of Food Additive Safety. I think it has been very successful. We have had great topics. I know myself, as an FDA toxicologist, I have learned a lot from all of these symposiums.

This is a public forum and it’s a way to discuss science. It is not really a way to talk about FDA regulatory and policy decisions. It is a way for us to inform the science so when we make these decisions, we are doing it on the best scientific basis possible.

This eighth one is called Safety Approaches for Young Children. It is an area of great interest to CFSAN as many of the issues we’re dealing with have a special toxicity to young children. It is being chaired by Dr. Elaine Faustman, who will be joining us by webinar, and Dr. Allen Rudman, as I said, from the Office of Food Additive Safety. And we’re fortunate to have not only Elaine, but Dr. Gary Ginsberg from Connecticut Department of Public Health, Dr. Susan Felter from Procter & Gamble, our own April Kluever, and then a roundtable discussion moderated by Dr. Peter Goering from SOT, former SOT president, now past president, but also a [inaudible].

This is the last colloquium for the year. We have exciting topics for next year. We hope you continue to join us. We will announce those soon. Now, some important administrative issues. The restrooms are outside. There’s a cafeteria if you go outside to your left. We will hand out questions. We will have a panel discussion at the end, so we will be handing out question cards for you to write your questions. And you can come to the microphone and make questions.

With that, I will introduce Peter Goering, who needs no introduction. He is the past president of SOT.

Welcome from SOT and Introductions, Peter Goering, PhD, SOT Past President, US FDA, Silver Spring, MD

Thank you. Welcome to everyone in the room and welcome to our, what we think are approximately 300 participants on the WebEx. It is a pleasure to represent SOT and commend the FDA for the collaboration with SOT. This has grown out of a memorandum of understanding signed between the two organizations about five years ago. This has been a very fruitful collaboration. The SOT Council had a meeting this week, and I was pleased to report that the Council highly endorsed these activities. They look forward to them continuing.

I am pleased to share with you the mission of the Society of Toxicology, and the reason I would like to share the slide is because I think there are parallels between the mission and the priorities of the Food and Drug Administration. The mission of the Society is to create a healthier and safer world by advancing the science and increasing the impact of toxicology. We know that in this room, the mission of the FDA is to promote and protect the health of the public. There are a lot to parallels there in terms of strategies. For SOT, one primary ones is to strengthen the relevance and impact of toxicology. One of the major priorities at the FDA over the last five to
eight years has been to transform toxicology to increase its value in making regulatory
decisions. The SOT is dedicated to developing and supporting toxicologists. And just like this
session today, the FDA, many of our centers have their own staff colleges and we are, FDA
strongly supports the development of its workforce. This is one of the primary reasons that we
put on the colloquia.

The SOT has a strong interest in expanding its outreach of toxicology and toxicological science
on the global level and we are aware of the FDA's international programs as well. I have tried
this button. Please advance the slide. There we go.

This is the eighth meeting we have had. Here are the topics, starting with the most recent one,
and there are a variety of topics related to risk assessment and safety assessment. I have some
information that we are pleased to share, who have been involved with organizing these events.
These seven colloquia have had over 1800 participants by the WebEx and on-site we have had
430 participants. This on-site number would be over 500, had we not been blizzard out in late
January, but a tremendous effort by the organizers and the speakers allowed 300 participants to
participate by WebEx. Each speaker was in their home. Betty Eidemiller was at home
organizing from SOT, the staff here was somewhere running the WebEx for us. It was a
tremendous success even though we could not meet on-site.

Here is some data that many of you know that each of the colloquia are recorded. The slides
and recordings are available at no cost on the SOT website. This is data about the downloads
and you can see the spikes that every time a new colloquia was held and an invitation to go to
the website, many individuals were listening to the recordings and downloading the slide
content. The poly hydrated oil was the first one. You can see that subsequent to that as new
colloquia came on board, more people were downloading this. This has over 7000 people
visiting the site or downloading the material. Even the last one on the Cramer classification has
over 2000 visits to that site. People downloading content. Not only has this, are these an
indication of success, but we have webcast that is worldwide. We have dozens of participants
from, we have participants from about a dozen federal agencies over the course of the series.
20 NGOs have had representatives participate. We’ve had participants from over 60 universities
worldwide. And also, 160 companies have had participants tuning into this, CROs, consultants,
and consultants.

This success of these colloquia is due to a large part the brilliance of the organizing committee
and the SOT staff and the FDA staff that helps put these on. Ivan Rusyn is not here but he is the
chair of the committee. And we have representatives of SOT and the Center for Food Safety
and Nutrition on the committee.

I would like to introduce Dr. Allen Rudman from the Center for Food Safety and Nutrition. He is
the chairperson of this colloquium, along with his co-chair, Elaine Faustman. So, Allen,
welcome, and we look forward to the session.

**Speaker Introductions, Allen Rudman, US FDA, College Park, MD**

How can I ever do better than Peter? Thank you very much. Good morning.

This colloquium is different than some of the others. Typically, we focus on toxicological
methodology or safety. This one, we are actually going to talk about children, not just the
methods but different types of methods involved in safety and assessment of young children.
I would like to introduce now, from afar, she is in California, she will be on the WebEx, Elaine Faustman. She is a professor in the department of environmental and occupational health sciences. She is the director of the Institute of Risk Analysis and Risk Communication at the University of Washington. She directs the NIEHS EPA funded Center for Child Environmental Health Risk Research. She has served as chair of the National Academies of Sciences Committee on Developmental Toxicology and is a member of the NIEHS NTP Committee on Alternative Toxicological Methods. She has published over 200 peer-reviewed publications and technical reports. If we can get her online.

Faustman: Hello, Allen. I think I am on. Can you hear me?

Rudman: Yes.

Children Matter: Using a Lifecourse Approach to Understanding Safety Assessment Needs for Children, Elaine Faustman, University of Washington, Seattle, WA

Excellent. Thank you so much for the great collaborations between SOT and FDA. This is great. I am very sad and disappointed I am not able to be there. I am north of California. I am in Washington State. I send my best regards. I am pleased to be able to share with you remotely.

The topic on what I will talk about today our children matter. I want to introduce, they’re kind of going fast. I am not doing that. We go back to the title slide for a minute? Okay. I will say next slide and then we can proceed.

The title of my talk would be looking at a life course approach to understanding safety assessment needs for children. The next slide shows that while I am interested in risk assessment issues for children, I have no conflict of interest. Should I proceed and do this? I do not seem to have control over the slides. Let's go on to the next one.

This is a slide that many use. It is to remind us when we are thinking about extrapolations and trying to understand relevant dosage, that we use scaling automatically. I think in this particular case, we want to think about but this, this is not elementary scaling but there are many features. I am hoping by the end of my talk, if I am successful, you will understand some of the nuances and how we think about that and framing issues for children safety.

Let's go to the next slide. I will do this by introducing some of the concepts of life source analysis. I will use the risk assessment framework across life stages. I will build on the social ecology model of development. Often, we do not talk about this but underlie how we think about these things. I am going to talk about how we organize and analyze data across life stages. We will also talk about what this means in a broader context.

Please hang onto your seat. Let's talk about this issue. I talk about an overall framework on risk assessment on children development. In this case, as many of you know, I am in a to see if of risk assessment approaches. You can see we have exposure, genetics, dynamics and outcome. In this particular case, I will discuss the neonatal and early childhood time period. That is what this symposium will be talk about. With other and future symposiums, we may talk more about in utero exposures or adolescents. It is very important to look at the same for work. We just need to have the specifics of the toxicokinetic and dynamic considerations that are relevant
for the age specific groups. We are going to talk more detail about each one of these parameters. Let's go on to the next slide.

Let's talk about the exposure aspect. Many of us have seen variations of the slide, the child specific behaviors can result child specific exposures. I love the slide because in many risk assessments, how much dirt a child eats by hand to mouth activity or how much dirt gets trapped on food that gets dropped on the floor and put into the mouth. That is an example of a child specific behavior.

If we go to the next slide, let's talk in more detail about this. There has been a lot of activity, college with the EPA have been monitoring a series of age specific children activities that can impact exposure pathways. In this particular case, this slide was generated by the EPA and it was used in the WHO guidelines. What it shows is that if you look across the different activities, they are activities like playing in the sand or eating stand by that occur at one place, your breastmilk exposures versus solid foods versus ideas of where children might be in day care centers. All of these locations and location specific activities can contribute to how we think about exposure pathways. If we look at this across ages, it becomes very important as we move forward.

The next slide relates in terms of consumption of food and beverage as a ratio of intake to body weight. As we go from six to nine months up to 16 to 64 years, the food consumption patterns can change dramatically. Knowing the age specific aspects becomes extremely important in our risk assessment and calculating the exposure of risk assessment for children.

Does this make a difference? I happen to pull out one set of slides from a larger slide deck that we have used and looked at from [inaudible]. We know that from national statistics, the cumulative problem does probability of being exposed to a metabolite in this case, or a representative of these compounds or residues that are present in food, but if we look at this probability for this metabolite in urine, adult or to the left and as you go to the right, you have younger children. You can see there is a pattern that children on this scale have higher exposure. We have great resources such as the [inaudible] survey. This confirms from a biomonitoring standpoint what we're saying from the exposure prediction standpoint. These factors go together.

This shows some of the tools that are available out there. As I work across the globe and talking to different groups on issues that are children specific behaviors and exposures, you begin to realize what a phenomenal resource this document is. Child-Specific Exposure Factors Handbook has helped define how much they eat in various stages, how much other exposure parameters are changing across life course. I always like to highlight this and realize that many other countries use our statistics, even though there is a very strong cultural difference between how when solid food is introduced to or different behaviors were kids. We have this resource and how to use it. Let's move on.

Recently I was very involved in the national children's study. One of the things that came from the work, it was the idea of how do we start to define exposure parameters across the life stage? How do we frame the studies to understand what happened in our population? I like to use the slide because it shows us that the data that supports the various parameters in the risk assessment frames, they are collected by environmental measurements and predicted for exposure for direct biological measurements which might be in urine or saliva or something like [inaudible], which is the first [inaudible] for children. It could be from observations and questionnaires. These move together to give us a more true reflection of our exposure. This
needs to be repeated at both the individual level, understand how households and the family considerations, and at the community level. Multiple scales are involved in is. We are looking at air pollution or water contamination. That can mold how the exposure is being looked at. Things need to be repeated across life stages.

We were involved in some of the issues around those issues and were called in from some of the [inaudible] to look at water consumption in high school. Teenagers just do not drink water. We do not have to worry about the large contamination in the drinking fountain because no one is using them. That is interesting. You have to see what is going on when we look at these risk assessments for different groups. Let's go to the next level.

Hopefully I have illustrated the idea to need to consider both, need to consider exposure. What about toxicological differences and dynamic differences across life stages? Let's go to the next page.

If we go back to the overall framework, when we look at this and we talk about exposure. What we want to do is we want to look at what kinetic and dynamic factors are changing across this life stage. Gary Ginsberg will be highlighting many factors of the kinetic pathway. We will hear about some of the implications of the dynamic [inaudible]. Let's take a look at one more level of awareness as we move forward.

What I would like to do is go to the next slide. Let's start to define the aspects. Many of you have seen this [inaudible] slide that talked about, what are the differences between children and developmental processes? This is the hallmark slide that explains in utero that the initial appearance of the organs and initial proliferation of the cells and the differentiation of the organs, defined windows of susceptibility in utero. We know it is extremely important to look at the three stages in utero. The first, second, and third trimester. Recent examples of Zika emphasizes these very early time points in the first trimester and even in an interesting window of [inaudible] development there that help us understand potential mechanisms of action.

What happens after birth? What kind of factors whether it is the same or not? I want to use a couple of examples of this. The example on the next slide is starting to talk about these key processes of neurodevelopment to remind us how this move in utero development where we need to consider in early child development. Dynamic processes going on including proliferation and migration and differentiation. These processes form the basis for different activities, different dynamic processes are going on in different organs and different layers of organs.

On the next slide, we can look at sensitive life stage and region-specific neurodevelopmental processes and using a road motto here. We have to be careful because there is a dramatic difference about what happens in models that we use for predictions versus human situations. It is even for other model systems. We have to look at the relationships through the processes as we extrapolate information from in vitro models to humans. But remember that this is not only at organ specific level but also a brain region level.

We have midbrain, cerebral cortex, cerebellum, and hippocampus. We see is that some of the activities of proliferation and migration, which are the two lighter shades on the left side, occur in utero in our animal models for midbrain and cerebral cortex. Cerebellum development for these processes of proliferation and migration occur postnatally. We also postnatally have a variety of processes going on for apoptosis. The idea of chemicals interacting with the specific processes in specific regions start to define the dynamic differences and challenges as we move forward and look at the processes.
If we go to the next slide, now, we plot out nervous system development in humans and rodents. This is from a paper that myself and several others put together. This shows that at the top and the bottom, both human and rodent comparisons. You can take various important components of the nervous system and plot it out as to where the events are occurring. It will give you an idea of what is happening gestationally, what's happening postnatally. And starts to allow us to extrapolate from the animal model predictions but also identified potential sensitive time frames will be. Will it be in early postnatal? Or later postnatal? Will it be in neonatal or infants?

We are talking about the dynamic processes. I want to remind us that associated with the dynamic processes, these are at the metabolism level. We will hear more examples of this when we hear and see Gary's talk. In this case, if we look at the one system of metabolizing enzymes, and this is diverse and broad. We can look at the evolution of the P450 isoforms in the human liver, we can identify enzymes that are active in utero. We can also see it in human or enzymes that have patterns of expression and activity after. We have neonatal groups such as early and late P450S. What is striking is the peaks that occur in early neonatal period. In some cases, it is after birth. If you look at this in the yellow, and you look at the field groups in 4A, some peak higher than adult levels in the neonatal period. Many of you know, then, that we have to adjust pharmacological doses, we have to increase various pharmacological doses for various compounds such as phenobarbital and others because of the rapid metabolism in neonatal. It is larger doses than what we would consider in adults. It is not always the slow onset of newborn or early neonatal metabolisms but in some cases, it can exceed adult levels. That means that there is a complexity to the rollout or evolution of these processes that requires us to understand the information as we move forward and think about safety assessments for this. This is a reflection of the cytochrome P450 system, but this is true for other systems.

I use an example, several other examples in a minute. One thing that happens is that it is true for transporters, and it is true for other enzymes. I have two slides that are a little bit specific. This shows that we work a lot with the organophosphates. We were looking at [inaudible]. This is organophosphate, kind of classic pesticide that many people study. You can see that activation of the [inaudible] is dependent on multiple cytochrome P450 isoforms. The breakdown of the [inaudible] is dependent [inaudible] to form an inactive compounds [inaudible].

If you look at the next slide, not are the are the cytochrome P450s having an evolutionary onset, but also PON1. Our colleagues [inaudible], looked at the onset of [inaudible] levels in children. They do not reach adult levels until five or seven years. This is beyond the neonatal and into the older childhood period. It is specific here. The implication of some of this work is that there are probably one in this and they do not become important until some of the later life stages. Again, hinting at the complexity and the layering of data that is needed in life stage considerations.

The next slide takes us back to the slide where we were looking at life stage and risk assessment. Hopefully I gave you some examples about exposure differences, whether they are exposure differences that are relevant for children and for neonates. In this particular case, I have given you some examples about kinetic and some examples about dynamics. I want to go to the next slide and talk about some of the testing paradigms that build on this.

One of the challenges in the area that I work in, in neurodevelopment and reproductive is that these processes are occurring, and as you can see, at various life stages. When we think about the spectrum of toxicological experiments that we need to understand differential impacts, whether it’s in utero, on behavior early on, behavior that results in alterations in the onset of
puberty such as adolescents, or whether we are talking about multiple generational effects, we need a portfolio of test systems to understand the impacts on chemicals across life stages. Some of the talks you will hear, some of the approaches for extended studies, and I want to put a slide in here to remind you that if we are looking at developmental toxicity, we will look at functionality common exposures that might occur early on and functionality that may not be revealed until we have challenge and behavioral challenge studies that would reveal the functional alterations. We also look at the temporal onset with reproductive and puberty completeness.

I wasn’t going to go much further than this but to remind you that these life stages and relationship with the appropriate study designs, determine whether a compound is causing effects are not, is also life stage specific. It needs to be inclusive on the earlier exposure as well as later onset of impact. Yesterday, I was on a call with WHO and people are interested in the origins of adult diseases. More and more, there are study models that are looking at exposures that occur in early childhood that may then predispose individuals for later events such as early onset of menopause or early aging or early narrow degenerative diseases. These have important implications.

The second consideration is on the next slide that I want to emphasize as we use more and more and be true systems. We can use experimental systems to assess and predict developmental impacts. I use the example for neurodevelopment. We need to understand the questions that we have we need to consider biological complexity. What can simple cytotoxicity assays and [inaudible] tell us versus impacts in neuro progenitor cells and neuro progenitor cells that might be in organoid like forms so we start to get three dimensionality to our cell systems, will that improve out ability to predict impacts on early neurodevelopment? And the answer, we know due to some recent work from predictive toxicology centers is that 3-D is better in many cases. It can answer more complex questions. It depends on your risk assessment question on that. I think the challenge and we will hear about this, perhaps even in another FDA session, how do we fully incorporate these dynamic assessments that are occurring in cells and three-dimensional cultures or in simple model systems and be able to predict the impact in humans? This is where the dynamics and toxicodynamics really come into play, in understanding what questions these systems are very good at answering, and what systems are still challenging for us? There’s a lot of answers [inaudible] looking at functionality in these systems, and the work with C. elegans behavior studies and zebrafish behavioral studies are certainly areas where there is lots of interest for predictions. It is not just that we have lots of different in vivo systems, we also have cell systems and organize systems that emphasize different processes and proliferation or differentiation or functionality. We need to keep those appropriate.

If we go to the next slide, we can look at the life stage framework. We have been talking about exposures. We've been talking about toxicokinetics and toxicodynamics. And we talk about the basic processes of metabolism and development change from in utero, postnatal, infant and adolescents. This is an aging population because of my own interest in aging. It also illustrates the concept that I just brought up, this idea that these early exposures, the manifestations [inaudible] may not occur until much later. We have narrow degenerative diseases that are a result of these early impacts.

The other reason for looking at the slide, is to think about the idea of population. Many times, [inaudible] we have an individual who, how we set up the guidelines to ensure protection across the life stage. For many of the, those in assessment, we were interested in the population level. What are the ramifications across the population? We talked about individual genetic variations
impacting the next metabolism profile. When you'd start to talk about the variations, it begins to get very interesting.

I will use one example on the next slide as an area where I think lots of emerging data, that we need to consider, and this is a, a title slide called life course consideration for epigenetics. We pull this from a 2009 paper. I like the layout of this. One of the things is the discussion about the early impact on epigenetic and programming various pathways. This is the underlying regulation of onset and paneling of the process that we have been talking about with early childhood.

We there is a differentiation and functionality here. The reason that this begins to get very interesting, we have organ specific issues. Work in this area and the programs of the testes was known about almost 25 years ago and was used as a hallmark of the onset of epigenetic programming. We have been very excited to see this. We looked at this from the standpoint of early exposures to the mother or the father, exposures in utero, but also looking at the events in early childhood just after birth and maybe modified again to provide an understanding for altered impacts on onset later in aging.

The other think that the slightest good about, it talks about lifestyles because these are additional factors that will help either suppress or express some of the epigenetic signals. I am not trying to make things more simple for you but to realize that there is a lot of activity in this area. We sometimes get isolated in fields as scientist, that we forget the public thinks about these things. In this particular case, I was at a meeting with a group of pediatricians. They were reporting to us that epigenetics has made the headline news. They were getting patients coming in and saying that they were concerned that their child had been exposed to epigenetic modifying compounds in the environment. They wanted drugs to be able to modify the epigenetic programming. Some of us, after we realized that our jaw dropped to the floor, realized that sometimes scientists are effective at communicating our new scientific findings but not so good at putting context around it. The idea that people would be taking epigenetic modifying drugs because of some environmental exposure makes us feel concerned. We have a lot to do that we know a lot about life courses and developmental processes. But what we are trying to do is maximize development and not do dramatic alterations in these processes either unintentionally or intentionally in this case with the idea of prescribing a drug to modify the environmental impacts.

The next slide, this was put out with principles of evaluating risk in children associated with chemical exposures. We put these materials together. The WHO will be looking at these issues. If you have one place to go to, this is a very nice place to go to get some of the basics.

I want to also talk about on the next slide, how we talk about some of the risk assessment approaches when we get down to modeling. Some of the chats subsequently, you will hear about NOAEL and looking about the effect levels. We are also going to hear about some of the benchmark approaches where we are taking and identifying an effect level, perhaps an effective dose. We will also look at how we can come up with safe doses. The idea or the importance of these factors, they cannot be understated for life stage consideration because in addition to the experience of normal factors that we think about as shown on the next slide, and this is some definitions of the NOAEL forces the benchmark does.

What we have is the idea that we are adding factors and there is lots of discussions about this. I used a traditional set of factors to look at this, whether it is the average or sensitive human, the animal to human or the LOAEL to NOAEL. We can look at these life stage considerations. These are all not considering the ideas of life stages. You will hear some discussion of this,
there are additional factors that have been discussed on general Food Quality Protection Act and the child specific uncertainty factors and the area of looking at early exposure and cancer effects, we talk about some of the age adjustment factors that are considered that look at both the timing of exposure and where that is in the life stage as well as the sensitivity of the tissue that is being exposed and what that means for the outcome.

My purpose is to give you a broad brushstroke about the factors. I will not expand on these. You will see some good examples and discussions of where we are and how we modify the factors moving forward.

To remind you, this is how we think about putting things together, exposure, toxicodynamics and looking at outcomes. This is related to exposure assessment, toxicity assessment, risk assessment. This has helped to form the basis of how we think about risk assessment as well as safety. Let's look at the next slide.

This is a slide that is from the WHO materials. It is in the yellow book that I was talking about. It shows you that across organ systems, whether it is respiratory, immune, or renal, the majority of our exposure and impacts data numbers have been generated for embryonic and fetal stages, little bit for infant and a little bit for child. As you go along with different chemicals, we still lack a lot of information in the area of infant, child, and adolescent impacts and assessments. We will hear more about that. That is important for what we are here about.

Let's go on to the next slide. I want to remind us, with the national children's study put some of their early framing together, this was in 2010. They went back and looked at pediatricians. If you were a nutritionist, if you were someone from physiology group, or if you were someone who was working on medical classifications such as [inaudible] up there, you see that everyone has different definitions for childhood and infants and neonates. What they did, and this is an important product of the national children's study, they were trying to harmonize the language and the [inaudible] used to even describe childhood and early childhood versus neonates. If you look at the pediatrics, the yellow line, that differed quite a bit from where you were looking at things like EPA at the bottom. Early childhood was one to four years in one system, and in other cases, childhood was just defined as two to 11. That is an important emphasis on terminology. In yellow book, the WHO also did this.

I want to remind you, this is a much larger context. We are thinking about environment and chemicals. Really in life course, as we have been more frequently defining it, is this really multiple interacting influences adjecting children's health. It includes chemical and nonchemical stressors, and children's health is the middle of this, where we have social environment, biology, behavior, and physical environment. And this is from a report from the Institute of Medicine.

If you look of the next slide, these factors in the environment change dramatically across time. This is the concept that is being used in this matter, to look at chemical and nonchemical stressors and a broader context. There is a very large literature that toxicologists forget to look at. I am encouraging you to do so.

I hope that emphasize the importance of early exposure. The complexity of factors, the complexity of environmental exposures that might be experienced, and that these differ across life stages. We need to consider these across multiple stages of development and at different levels of assessment. Are you talking about changes that are occurring biochemically? Are you looking at molecular or functional level? We need for framing risks and I believe that the risk assessment framework allows us to consider the life course aspects. We also think the life
course frameworks allow us to look at chemical and nonchemical stressors and the idea of nutrition as well as the context for our chemicals of interest as we move forward.

This is an acknowledgment to many people, including the NIEHS, [inaudible], EPA, and also early on, some funding from FDA. I want to end here and to remind you that in the slide deck, there are some references that we used quite frequently. I referred to those in my talk. Thank you very much.

Rudman: Thank you. You did a phenomenal job. Are there any questions in the audience? Please come to the microphone. That way everyone can hear you and she can respond. These are clarifying questions, please. No one?

Okay. I will go on to the next speaker, this is Dr. Gary Ginsberg, who will be talking about Early Life Development of Toxicokinetic Pathways: Framework Case Examples and Implications for Safety Assessment. He is a toxicologist in the Connecticut Department of Health and Public Health Division of Environmental and Occupational Health Assessment. He is involved in the use of toxicology and risk assessment to evaluate human exposure in chemicals, air, water, and etc. He has published in toxicology, carcinogenesis, pharmacokinetics, and other areas and he is in an adjunct faculty position at the [inaudible] School of Medicine, as an assistant clinical professor at the University of Connecticut School of Medicine. He served on the US EPA Science Advisory Board and numerous other boards. He has received a PhD in toxicology from the University of Connecticut. Dr. Ginsberg?

Early Life Development of Pharmacokinetic Pathways: Framework and Case Examples with Implications for Safety Assessment, Gary Ginsberg, Connecticut Department of Public Health, Hartford, CT

Thank you. I will get the microphone in place. Right. We have heard from Elaine that doing risk assessment on children can have challenges as many different components. We cannot test chemicals like we can in rats and mice in children. We have to be as predictive and thoughtful about this as possible. How can we use the data that we have, whether is in vitro or animals, whether in pharmaceutical, we try to do the best job possible? From my position as a toxicologist at the state health apartment, we see children exposures all the time. The question comes to us from parents, from community leaders, etc., what about the kids? What can we say about different areas of exposure or their toxicodynamics? Of course, that's not a word I would use with the general public, but certainly one we can use here, to talk about what's the effect of the chemical on [inaudible] or the toxicokinetics which is something of an area that I'll talk about, which is something that we can do a pretty good job with, more so them with some other areas. We have an idea of the [inaudible] of the metabolites in the systems and the [inaudible] of the body components that were chemicals and to reside. We can make some predictions, some of those are actually [inaudible] with pharmaceuticals that help us understand how to do this as good as we can, connecting the dots from the data sources that we do have in this particular area.

I do not have a conflict of interest, but I do want to say two things. One is that some of the work I will talk about, which I am author on, have been funded by the US EPA various offices that I have had collaborators with. Also, my talk in no way reflects the official opinions of the state of Connecticut or the US EPA.
What I want to impress upon you, are two basic things about chemicals and toxicokinetics. It is all about the speed at which it occurs, or we say the rate. There are competing rates, for example, absorption of chemical, if the rate of absorption is slow and the rate of fecal elimination is fast, you will not absorb much of chemical. Competing rates, and we have to understand the rates, so that we can see where chemicals tend to go.

The other principle beside rate is partitioning or dissolving. Where do chemicals tend to want to be in the body? Where will they partition into? Are they fat soluble and stick around for a long time in the body? Are they water-soluble, stay in a central compartment and we’re talking about distribution with the partitioning, with the rates. We are talking about everything from absorption to metabolism and excretion. Toxicokinetics, I like to tell my students, it is what the body does to the chemicals, how we absorb it and how we distribute it, how we changing it chemically, called metabolism, or how we excrete it. And toxicodynamics, we like to think about, it what the chemical does to the body.

So, these are very much background type information. We will move on to a little more detail in some of these areas. We will not spend too much time, actually just this one slide, on prenatal but it's important to keep in mind there are important issues toxicokinetically in the fetus. And Elaine did show some of these development, [inaudible] of certain cytochrome P450s in the fetus, which are looking very different than postnatally. But in the prenatal situation, the reasons why toxicokinetics are important are you get three different versions of exposure. One is the [inaudible] compound coming from the internal system, metabolites that are coming from the internal system, and then metabolites from the fetus itself.

For example, Elaine showed this slide where there is a group out of France showing [inaudible] is a fetal version of [inaudible]. [Inaudible] is a cytochrome P450 that handles a lot of therapeutic drugs. Fetuses don't have any of this to speak up what they have is a fetal version of it called [inaudible] which doesn't do exactly what it says. There are differences in rates and on that. So, it’s important to able to understand what the fetus liver could do versus what the postnatal or mature adult liver can do if you want to try to understand how chemicals respond differently in different systems. I encourage you to look at some of Lucy Anderson's work from the mid-1980s. It’s not hydrocarbons that I have on my slide. They did some genetic back process and the actually had [inaudible] side-by-side in utero which had different genetics, different aryl hydrocarbon receptor responsiveness. They showed it was the genetics of the fetus that govern the cancer rate from the exposure to the mother. So, the greatest risk was if the fetal liver [inaudible] was AH receptor positive and the mother was AH receptor negative. So, there is issues, whether it’s cancer or other types of epigenetic responses, metabolism matters.

And then of course in the postnatal period the rates of absorption could be different. For example, we will recognize the example that the rate of uptake in the G.I. tract is greater not just if they don't get a lot of things which we know is a risk factor for let absorption but just because the processes and the rate of G.I. clearance is different in early life than later it can affect the absorption of chemicals. Distribution is often having we think of these happy bouncing babies follow up lipids. If you actually drop it, you will never be forgiven for that. But you think this baby will balance. Actually, right out of the womb, maybe some have a lot of lipid. So, the partition of chemicals into body fat will be less coming right out of the womb where is the content of water in tissues is higher. So that will affect the distribution of chemicals. Also, babies don't have a lot of protein binding pics of chemicals that tend to be in a central compartment of the blood system can to have less about and it may be easier to displace a chemical the chemical interaction or drug interaction of protein binding.
Then of course another hugely important factor is the immaturity of the blood brain barrier both in the fetus an early postnatal, which allows heavy metal to get into the brain and not get back at the brain and you get messed up positions of mercury and lead that can develop very early and that opportunity goes away as that barrier develops.

And as I will spend more time on the rest of this as Elaine has already talk about, many cytochrome P45s, the acronym, the short name for that is CYP. Many of these metabolizing enzymes are immature and by immature, I mean they are not hardly expected. The genes are all there. But there is not a lot of expression, for whatever ontological reason, they are not expressed either in utero or in the early postnatal period. But each one of those, and I will show the slide on this, each one of them comes on board at different rates. Urinary excretion has the kidney functional but in utero you don't have to do much. The same thing with the lungs. So, the postnatal development is hurry up. Let's get this system going. But the willful to the kidney is not really, it's pretty low right out of the gate and then starts ramping up by six months. I will show you some data on what those rates look like. And then Phase II metabolism is glucuronidation, sulfation, [inaudible]. These conjugation pathways also come out of the gate being immature or slow.

Here is another overview where I talk about a variety of these factors. I don't have time to go into detail, but body composition and what are the implications of toxicokinetics in the right column there. With lower body fat it will be less partitioning and protection of lipid soluble chemicals and a larger volume of distribution for water-soluble chemicals. Actually, we think of deliveries been immature however, the liver mass per body weight is larger in infants through the first couple years of life. So, you actually would be getting faster metabolic clearances early. But it doesn't happen that way because the metabolic immaturity.

We talked about the immaturity of the enzyme system and I will talk more about that. The brain is actually larger per body weight, you know how kids seem to be top-heavy with these large heads and small feat they look like they are ready to tip over. By the brain mass actually is larger per body weight in the first month of life, and you have greater blood flow to the CNS as the brain develops. The permeability is greater and so the brain is more of a target for nutrients and also for toxic chemicals in the first month of life. Immature renal function in little bit of protein binding which are already talked about all have implications in the right column as I believe we will hear more about.

Okay. So, the caveat. Just because a metabolizing enzyme like a CYP is immature and low expression, it doesn't mean that greatly falters the state about chemical and the metabolic clearance of chemical, because there could be overlapping enzymes. There is a whole family of CYPs. And they have overlapping abilities. So, if one hasn't developed yet, under the one may be on board and may be able to partially or completely handle that process. And then there is another toxicokinetic technicality and that is that what is the rate limiting step. It may be that you have got plenty of enzymes there to handle the metabolism of the chemical that presents itself to the liver, but that the next step is how quickly is the chemical getting to the liver or recall that slow limited rather than compressive limited metabolism. So, if it's slow and limited that is just a matter of a function of how much blood flow is there to the liver rather than the capacity of the liver even in immaturity. So that is something, that another physiological and metabolic differences in early that could affect what we think of as an immaturity that must have a big effect may actually mute the effect or may enhance the effect. So, that is why we need to do physiologically based toxicokinetic modeling, so this is a predictive tool that normally allows us and was classically developed to go from the animal and what they are really shooting for here is what is the internal dose of the key toxic metabolite or the active form of the chemical. So, we
want to know in the animal study what the internal dose is that created the effects and then how much actual exposure in a human or an adult is needed to get to that internal affect level.

So that is what toxicokinetics has traditionally been able to do is extrapolate from animals and humans, so you don’t have to have a generic uncertainty factor, or a generic scaling factor associated with that animal to human of separation. Why not do that for children or for the elderly or for genetic polymorphism? Some of the research I’ve done with EPA has been on exactly that topic. Using PBTK modeling to extrapolate to the immaturities in early life or to the genetic polymorphisms that are evident in certain enzymes. What are the implications for internal dose because of those metabolic differences?

So how do constructive PBTK models work and what is it? You do need some relatively advanced simulation software. So, it's not something that anyone can just call up on Excel. It’s not as simple as doing Monte Carlo analysis. You [inaudible] layer Monte Carlo analysis onto a PBTK model and make it even more complicated but more distributionally predictive which is a great thing but you can understand it and you can sort of get your mind around the implications by understanding toxicokinetics mechanisms and how are chemicals handled by the body and understanding some of these metabolic differences across life stages. You might get at least a begin understanding of what a PBTK model may produce. I will go through a framework later that helps you decide what really is the priority and what should be the PBTK model and what do you want to put your resources into and spend the efforts on modeling versus what probably isn't a big issue and maybe you can make some other judgments about.

So, a PBTK model is basically taking the body and dividing it up into a series of compartments that have a certain size. And these are physiologically relevant sizes. We know how these organs change in size over time, and we know the blood flows to these organs. So, if you have an amount coming, we know the size of the organ and we know the water content and lipid content and something about the partitioning of the chemical in the tissue, so we can come with a concentration of chemicals in each of these compartments, which are physiologically relevant compartments. So, lungs, kidney, muscle. Why do we have muscle? Because muscle is the largest compartment in the body and so you have to account for it in these models. Fat of course you want to touch on because not much metabolism happens but it's a place of storage. And then the liver. If you think about water coming into a bucket how is water getting out of the bucket is basically what these models are trying to tell us. And the way it's getting out is through renal clearance and the chemical can exit the model through liver metabolism. We put all of the metabolisms to simplify things in the liver although we know that is not exactly true. But by and large is generally true.

And so, what do you need to have made these models? You need to have physiological constants again. We have databases on that and I thought the next slide would be databases, but I guess not. I will show this slide a little bit down the road with a little bit more data on these things. But the chemical properties with the absorption rate usually backed up which means you are taking purple data and you actually have to say what's the best number for that parameter that fits the data. For partition coefficients you can do that and figure out what the partitioning is of a chemical into these different tissues.

Plasma protein binding is a little more complicated to provide. The metabolic rate constants can come from and beach of experience with the liver or with systems just understanding the CYPs and how they are functioning, or it could come as a backlit with urinary excretion rates often backs. So, in these models they are the best predictive tools we have some as we have two or three parameters which just great and look uncertainty but when you need to do is be able to
calibrate the models and validate them against independent data, so you know they are predictable.

Examples where early life toxicokinetics matters, immature glucuronidation, well, we know that babies right out of the womb tend to be jaundiced, look a little yellow, what’s going on there is that their glucuronidation hasn’t ramped up to handle the waste product from the blood cells [inaudible] bilirubin, that they can’t really clear bilirubin as quick as an older child and so there is a metabolic and a fecal clearance problem. There is grey baby syndrome [inaudible], and again, slow glucuronidation of this antibiotic and it has led to grey baby syndrome or [inaudible] because it wasn’t being properly glucuronidated and cleared. Immature carboxylesterases [inaudible] that has been shown to be greater in juvenile rat as opposed to older aged rats.

Here’s some data from Timchalk et al., 2006. The postnatal day five is the top chart and these are two different doses, the open symbols versus the closed symbols, the closed symbols 10 times higher dose and the percentage of control [inaudible] we know that this is the chemical and that when it inhibits the brain it is having a toxic effect and the in addition is dramatically greater in the postnatal day five rats compared to the bottom graph with postnatal day 17 rats at the same dose given on that same day. So yes, these things do matter.

Now I am not advancing. Let's go back a slide. Should I be pointing this differently? My clicker just stopped working. Okay. That worked. All right.

I talked a lot about neonates so far but in older children the liver is larger providing, you often get this rebound of greater clearance than in adults in somewhat older children, so one to two years of age. Because again, the cytochrome P450s are catching up to adult levels and you are getting increased lipid mass, so you’re getting a greater ability to store compounds than in the infant.

Here is some databases that you may want to refer to. So, the physiological parameters ILSI 1994 publish a cross species, a nice database, and the US EPA updated that in 2009.

Now, what about the enzymology? Not in terms of the metabolism there have been a lot of data coming from liver banks. These are cadavers and specimens with either the fetus not making it or there was a child that died early in different ages. So, there are reservoirs of liver tissue from these cadavers that you can study. The ontogeny either through mRNA levels or through protein levels, different CYPs have been discovered. And then there is a number of these references with these kinds of data.

Then I will show you a little bit more of the work that we did in the early to mid-2000 and looking at the pharmaceutical data sets knowing that there are drugs that are cleared by hallmark classic metabolizing pathways and you can learn about the ontogeny of those pathways by learning about the differences between early life and older in terms of experience. The actual data we have for those drugs.

And so, this slide shows that you have got children being those and pharmacokinetics are used to understand what does global to use and it's a fairly rich data set on the children's pharmacokinetics at all different ages including very early in premature infants. And that we took that information and put it into an ontogeny database that we used to make some predictions for a modeling. So here are some of the results from that. So, the Y axis is the ratio of the children's half-light to the adult half-life for the chemicals involved. Here is the 40 drugs substrates we had data for and we go from premature in the first bar all the way out to adolescents. You can see
that the big difference relative to adults also a positive function is about the adult line and it's longer half-life with a slower clearance. Does everybody understand that?

So, the premature neonate was four times slower and a longer half-life than older ages and you can see that catching up so by six months you were actually bearing the infant factors. Regarding specific properties, we had about seven antibiotics and they were not clearance upon renal elimination, no liver metabolism involved. So, their clearance is a good indication of how well the kidney's functioning at early stages. You can see there is about a threefold difference from adults in the earliest stages and to the next catches up as blood flow to the kidney catches up.

Why do we care as a toxicologist about CYP1A2? It is involved in the metabolic activation of [inaudible] that you can find in food or that, like, Benzedine it is in some dyes that children may get a dose of when chewing on clothing although many have been taken out of manufacturing. So CYP1A2 is important to early life. And you can see that now we are not are talking about tenfold in the full-term neonate compared to adults in terms of slower clearance.

These are glucuronidation substrates, and you can see four or more-fold different in the premature neonates, but threefold different in the full-term neonates compared to adults. And we look at variability, one concept is how different are kids on the average case and then what is the variability in early life? This window is many days. It is that we are going out years. So, the timeframe is truncated for early life here. But you can see the huge variability among the Y axis in the earliest timeframe that comes on with developed. So, variability is another important part of this.

Now are there other systems or enzymes that we care about in toxicology? It is CYP2E1 because it's involved in the insololvency and it's very important toxicologically. You can see it looks like a protective factor because in the first weeks to months of life, there's not a lot of CYP2E1, and it does take a while to develop.

On the other side you say what is the detoxification in early life and we don't have a good content data of the human liver in early life, but we do have is the city at a Puerto Rico which looked at plasma glutathione levels. It comes from the liver. We don't know all of the processes for governing plasma glutathione, but chances are it's a reflection of what is in the liver and 10% of adult levels in the first week of life and it's really fairly slow to develop. So that suggests a key biochemical defense mechanism would be in the liver at least in the plasma. And that is also important for detoxification.

I love this slide because it shows you some of your favorite enzymes are there but it shows you that the ontogeny and the developmental frame for these is highly variable and so you really have to know the metabolic mechanism of action for your chemical. And also, which enzymes are involved in that metabolism to then be able to use this chart. So, if you know the MOA you can start getting into the differences and start modeling it.

So, some of the issues I will talk about now is distribution. So, when you are thinking about toxicokinetic MOA, it is important to think how is my chemical I'm trying to understand how is it distributed. Is it highly protein bound? There may be more free toxicant available to do whatever damage in young children that don't have a lot of fat or protein binding. So, they might tend to be more free drug or free chemical in the first month of life. Metabolism. Is a chemical activated or is it detoxified and which enzymes are involved? And also, excretion and the pathways and how is my chemical handled [inaudible] go through these pathways?
So, if for parent compound active using the example of Toluene this is a great study in 2006. This solid square, I think I can point to these with my mouse. I just lost my slide. Having trouble clicking again. Okay. Now it wants me to get out of this. Okay. So, the squares show the actual data in adults. And all of these lines show simulations based upon how much CYP2E1 is in these various children and the inhalation exposure that stimulated these children. You can see three or greater blood concentrations are being predicted by the PBTK model compared to the adult.

So that is an active parent compound. But if the metabolite is active you might get as much, you might get less sensitive in the cases of acetaminophen or early life to have higher internal dose and the example would be the deficient [inaudible] or the organophosphates having potentially higher internal doses in early life. And maybe similar to adults in the example is Acrylamide.

Here is a paper we published on Acrylamide. Of course, everybody knows that Acrylamide is in a lot of food products and it's not the parent compound so much, Acrylamide itself, but it's the active metabolite [inaudible] which have to go through CYP2E1 metabolism which is immature. And also, it's also immature with the hydrates down here. So, you have got these offsetting in maturities. We found that the area current or the internal dose with the modeling for Acrylamide itself it could be three times higher than the adults and five times higher if you consider the variability of children. So, if you go to the 99th percentile of the children's profile compared to the median of the adults, you are about five times different in terms of the compound that is a little bit less because of the offsetting in maturities for the active metallic Acrylamide is two times greater in the child's system but five times greater with the 99th percentile. Suggesting that yes, if little babies, if they neonates are eating French fries, they would get a greater internal dose. But fortunately, by the time they are there metabolism has ramped up.

In terms of the uncertainty factor that we normally consider for this early life window, I'm sorry, not of the early life window, but the uncertainty factor for toxicokinetics in risk assessment, we usually think about the inter-species, or across human beings, as being 3.3-fold different. If it's within 3.3-fold different, then we say, we could safely use that traditional default certainly factor and basically we have got it covered.

And if you look some of the data I showed you on these metabolizing systems or just the toxicokinetic example of Acrylamide, you are within that threefold difference when you look at the average case. But when you are out here and looking at, I cannot really operate this. Okay. There it is. When you look here at the 95th and 99th percentile to the adult mean, the blue is the adult, toxicokinetics and the internal dose for the child would be about here. You might have a threefold difference in the average case. But when you are looking at the variability in the child plus the difference just because of the age, you might be sometimes five or 10 times different when you are considering the variability and the ontogeny difference.

So, this is paper by Renwick and Dorne, European regulators and toxicokineticists. They point out as well but when you are looking at, here is a variety of things with metabolism pathways for children and adults. Just take CYP3A4 here. Let's look at that line. And the geometric mean difference within adults system the default uncertainty factor in this assessment. But you when you're looking at the 95th or the 99th percentile the difference is more differential. So, they met the point in related paper in Tox Sciences when you're looking at variability you might want to be thinking about an extra uncertainty factor for children's toxicokinetics.
Okay. All right. We also publish a series of papers with the Office of Children’s Health Protection on differences in particles of symmetry. Of course, children’s breathing rates are greater plus the surface areas along the respiratory tree in the upper regions and in the deeper thoracic regions is different. There is a lot of postnatal development of the lungs and when you look at data that is available in the literature and we did some modeling on that the biggest difference we saw was about 4 to sixfold in terms of practical symmetry.

The X axis shows particle size and the Y axis shows dose per service area in the pulmonary region. And the three-month-old child is simulated here versus the adult here. You can see the difference is greatest in the fine particle region suggesting that babies will get a greater dose of fine particulate matter that what the adult.

Okay. All my red light is on, so I would just introduce this topic were moving onto. You could build toxicokinetic models to do all sorts of things. But the question becomes how do we scope this and how do we do a better job of thinking about the implications of what we already know about these properties in terms of applying a qualitatively coming up with an understanding of the degree of uncertainty we have got going into the assessment and the need to do PBTK modeling. So, our data is available to scope it and also actually populate a PBTK model. How much can we say without actually doing the modeling? I have a framework list of issues here that one can consider in scoping early life toxicokinetics. And what we will publish some of these case studies that I don’t have time to go through. But acetaminophen with different cases highlighted in that example with also each one which we plan to show.

I just want to finish up with my summary. Early life to adults internal dose differences, the size of those differences, are probably going to be case specific. But if you need to come up with a generic uncertainty factor to cover that difference, the one we use in risk assessment all the time, threefold toxicokinetic variability factor, is probably reasonable in the average case, but it may look more like five to tenfold when you think about child to child variability. Therefore, we really should be, because that toxicokinetic uncertainty factor may not cover all children all the time, we really should think about they need to do PBTK modeling and there are ways that we can try to understand through understanding the toxicokinetic mechanism of action, how the chemical is distributed and metabolized and what are the ontogenies, what are the immaturities, in those pathways and in those systems, we can start making some predictions about might a PBTK model show us and prioritize which chemicals we really need to do PBTK modeling for. Thank you very much.

Rudman: Great talk. We have time for one clarifying question. Is there any? No questions, then. Then we are going to take a 20-minute break and return at 10:30. Thank you.

Break

Rudman: Okay. Welcome back. Welcome to the second half of the colloquia. I would like to remind everybody there will be a panel discussion right after this second speaker. Please stay because this will be a very interesting thing where people can ask questions, even on the web. Please stay for the panel discussion.

I would now like to introduce Dr. Susan Felter, a research fellow at Procter & Gamble’s Central Product Safety organization. Her primary interest is methods for human health safety and in her corporate role, Dr. Felter leads the global teams responsible for the company’s methods for human health risk assessment for cancer and noncancer endpoints, providing guidance across
all geographies and business sections. She has a responsibility for internal methods to ensure safety in Procter & Gamble's baby care organization and she currently serves on the Science Advisory Board of the Food and Drug Administration’s National Center for Toxicological Research and the chartered Science Advisory Board of the EPA. Dr. Felter holds a bachelors from MIT and a PhD in toxicology from the University of Cincinnati. I would like to welcome Dr. Felter and her talk on Ensuring Safety for Early Life Exposures: Adequacy of Current Methods and Opportunities to Advance the Science. Thank you.

Ensuring Safety for Early Life Exposures: Adequacy of Current Methods and Opportunities to Advance the Science, Susan Felter, Procter & Gamble Company, Mason, OH

Okay. Thank you for the introduction. I had some notes here. They appear to have been stolen by our moderator. Very sneaky. Thank you. Thank you to both the FDA and SOT for inviting me.

I will start by recognizing I am employed by the Procter & Gamble Company and we manufacture and sell a number of consumer products used by infants and children such as diapers and wipes. Most importantly, you can see the artist who rendered the sketch of the first ivory baby was not aware that infants and children are not just small adults and you'll be pleased to know artwork has evolved along with the science.

When we talk about infancy and childhood with susceptibility they often think about the inherent of sensitivity of the exposure to chemicals. I would emphasize how important it is to consider exposure as well. Then I will talk about that which has been widely accepted for establishing exposure limits and whether those methods provide adequate protection for early life exposures. I will talk about opportunities we have to refine assessments based on default assumptions including the use of PBTK modeling to look at life stage differences and finish with a case study on phenoxyethanol which is widely used in consumer products.

I want to first distinguish between various terms that are used to describe the potential for individuals experiencing higher or lower risk over all to chemical exposure and I will note these terms are not used consistently in the literature. You see publications that define them as the exact opposite which happens fairly often where we don't see that consistency. I'm using the terms as defined by the US EPA. Sensitivity is a difference in responses aside from toxicokinetic and/or toxicodynamic differences. So, these are sort of the inherent physiological differences between individuals and including life stages that contribute to risk. Susceptibility includes the risk resulting from variation in toxicity response and also exposure. And then vulnerability includes sensitivity and susceptibility but then also includes extrinsic social factors such as violence or access to healthcare which can impact one’s overall risk profile.

Let’s start by considering exposure, which can be qualitatively and/or quantitatively different in infants and children from adults. I think this issue was really brought into the limelight by what is now called the Sentinel report by the National Academy of Sciences 23 years ago on pesticides in the diets of infants and children. This report provided a review of the state of the science at the time for understanding whether infants and children were more susceptible, and one key conclusion of the report was that centered on exposure and found differences in diet were generally more important than age-related differences in sensitivities in terms of defined overall risk. Young children often have a higher intake of common fruits and vegetables and maybe not as in the case of broccoli. The point being diet really can be different and it can be higher or lower. This really is something that can be a significant driver of differential response.
The NAS went on further to conclude children may be less sensitive than adults and the quantitative differences in sensitivities between children and adults are usually less than 10-fold. Five years later the European Scientific Committee on Food issued a report on similar topics. They recommended that a special daily intake was not needed in general for infant children, but that because they have a higher intake of some food items that that should be exquisitely considered in the risk assessment.

It is well known that infants and young children have a higher exposure per kilogram to food, water, and air than adults do. I actually saw this on some news program just a couple of nights ago where they cited a six-month-old infant is about 1/10 the size of an adult in terms of body weight but has one third of the caloric needs. They are growing so quickly so they do have a higher intake.

The same can be true for dermal exposures which is an area I spend a lot of time on. Here we are looking at the skin surface area to body weight ratio. Let me see if I can make this mouse work. We all trying to make the mouse work and it's a little tricky. I might not be pointing a whole lot. Sorry. They can see there is at first about a 2 to 2.5-fold higher skin surface area to body weight ratio between infants and adults. This goes down so by the time you are looking at a five-year-old child it's only a 1.5-fold difference. In the grand scheme of risk assessment twofold is often not very big. However, this can be really important if you are talking about an exposure in the NICU and talking about a pharmaceutical where we already have a tight margin between safety and efficacy. Sometimes this difference is trivial and sometimes it's critically important.

I will say that there are two general ways that if I am speaking about the world I come from, there is two different ways that we could address the difference between infants and children or adults. One way would be to extrapolate. If you have data on adults for the use of something like a body lotion and how much is applied, you could extrapolate to infants based on what you know and the difference in the skin surface area. The other way is to have data in the population of concern. Understand how your products are used and understand what the bodyweight, surface area, etc. are so your exposure assessment is specific for that population of concern. That is generally the approach we use.

So then when you do have the exposure assessment already intended for infants and children, it is no longer an uncertainty in the risk assessment and no longer an area of extrapolation and it’s no longer an area where the children potentially have the increased vulnerability because you’ve already accounted for it.

This slide I pretty much already talked about that we would consider the habits and practices of how products are used by infants and children and recognize a six-month-old could be very different than a three-year-old. Some of the physiological parameters that we would typically look at are bodyweight and surface area and one of the challenges is that these are rapidly changing in early life. So there is a need to, unless you have a life stage PBTK model, and some of those are being developed but most of us do not have those for all compounds, you also need to choose some values that will give you sufficient conservatism so you are protective in your assessment and also pragmatic because an infant grows so quickly.

Since I have been talking about dermal exposures, I will acknowledge the potential for dermal penetration which can be an important part of the overall assessment. I highlighted here, it’s the first part of PK in the ADME, the absorption distribution. So, technically speaking, absorption is part of pharmacokinetics. When we talk about dermal exposures it is not uncommon for some to
factor into the exposure assessment and that actually look at the absorbed doses. You will see it dealt with either way.

Questions are often asked about infant skin and how do the barrier properties compare to adults. The skin of infants does continue to mature for quite some time after birth. But in a full-term infant, the outermost layer of the epidermis that serves as the primary barrier is already histologically mature at birth. Importantly, it exhibits barrier function that is comparable to that of older children and adults. I have listed a couple of papers here that have looked at barrier function according to a few different ways and the overall conclusion is that while there will be some differences in skin certainly from infants to adults to older people as well it continues to change as we grow. The terms of its impact on how to risk assessment in terms of it serving as a barrier for dermal absorption the skin of a full-term infant can be considered to be comparable to that of an adult.

So, if I am doing a safety assessment for a dermal exposure, in addition to the potential for the more traditional types of endpoints that we look at in risk assessment, we also have to consider the potential for site of contact effects. Primarily dermal irritation and sensitization. So, the framework for how these endpoints are evaluated is very similar but the dose metric is different. Instead of milligram per kilogram bodyweight, you would look exposure in units of micrograms disbursed [inaudible] centimeters of skin and the assessment would be very similar in terms of comparing a human exposure to a no effect level that you have.

So for site of contact effects on the skin, we also have the added advantage of being able to confirm safety with initial safety evaluation on paper but that can also be confirm by critical studies that are conducted an adult. These can include actual clinical studies and also in use studies were people use the product. One of the questions is if you have some kind of a confirmatory study in adults, how does that provide adequate assurance for infants? And especially for something like an allergic reaction.

This data is for dinitrochlorobenzene which causes a type-4 hypersensitivity reaction, or an allergic contact dermatitis, in almost all people who are exposed to it. You can see in the graph there is a clear age-related progression in the reaction rate which is a reflection of the reduced capacity for cell-mediated immunity in neonates. The reaction the first couple of weeks of life is down around 7% by the time an infant is three to four months old you are already up to 62% to 75%. For an adult it is virtually 100%. This provides some assurance. This is data on one chemical, but this general phenomenon of infants being less susceptible to allergic contact dermatitis has been shown many times. It provides general assurance that critical studies done in adults for this endpoint will be relevant for evaluating safety in infants.

Turning now to systemic toxicity. We can consider whether our default approach provides assurance for early life exposures by evaluating the accuracy of the tenfold default uncertainty factor these for inter-species. This slide is called inter-individual differences. That tenfold factor is subdivided into factors for toxicokinetics and toxicodynamics. They are considered to be roughly equal in terms of defining the overall human variability. So, we can start to address the adequacy of this default uncertainty factor but considering what we know about overall human response and susceptibility and what we know about each of those two components. Especially for toxicokinetics where there is a rapidly growing body of research and literature. Some of my next slides has overlap with what Gary presented before. I will go to them very quickly.

Gary talk about absorption and a mentioned about dermal absorption. Distribution he also talked about differences with infants and children including the fact that an infant protein binding is
usually lower. But I will just mention one of the things that offsets that. For a compound to be excreted in urine, only unbound chemicals will be excreted that way. So, the fact that the lower protein binding can actually contribute to a faster excretion of a chemical. And I think Gary pointed out many examples where there are competing processes, one of which might increase sensitivity and one of which might decrease sensitivity all happening at the same time.

Gary also talked quite a bit about metabolism. This is probably the area where which we have the most information and it's quickly growing as well. We know that some are polymorphic maybe there could be genetic polymorphism that leads to individual differences. This is true for adults as well as children. This concept of life stage variability is superimposed on top of genetic differences that persist for a lifetime and not just during early life. But it's also recognized that even though individual enzymes might have factors that go beyond that 3.2-fold default uncertainty factor this does not translate necessarily to the same overall sensitivity. Gary mentioned the same as well. Metabolism can be multifactorial and certainly that is true in the field situation that Elaine talked about. There can be other factors such as hepatic blood flow which is sometimes the rate limiting factor. It's important to recognize that those differences can be measured in vitro that is specifically looking at CYP activity may or may not have an impact on the overall assessment.

I just wanted to show a little bit of the data from the impact of hepatic blood flow from Nong et al. This is the ratio of hepatic blood flow to liver volume and hepatic blood flow to body weight. You can see that for the neonates less than one month old, in both cases that factor is quite a bit higher so there is much greater blood flow to the liver when it is normalized to liver size and/or body weight, and the liver size is also much greater in a neonate per kilogram body weight. These factors together can significantly offset some of the immaturity of the hepatic enzymes seen in early life.

This summarizes some of the key points from a review paper by [inaudible] Dorne et al. where they looked at the age-related variability across the human population and they found that 3.2-fold factor for toxicokinetic variability was quite conservative when considering a healthy adult population. They had data specifically for children and infants on 10 different pathways and neonates for 5 pathways. The five pathways I have listed are the ones with which they had data. The majority of the pathways related factors for children were well below the 3.2-fold factor. But they were higher in neonates. Gary actually show some of that data.

One thing that really confounds our ability to know what to take away from this is the data from neonates was from pharmaceutical studies involving intravenous exposures where the data from children and adults perform oral exposures. As he got into modeling and understanding the impact of dose rate and how quickly a chemical is delivered for example that difference in dosing can have a significant difference in outcome.

The last major bucket for the toxicokinetic piece is excretion and the major route of excretion for toxicants via the kidney, which increases, so, the kidney function is really dependent on renal blood flow which Gary also mentioned is low in early life and then increases significantly following birth as a function of those increased cardiac output and a decrease in renal resistance. There are some examples especially of young infants being less sensitive to a renal toxicant specifically because less of a chemical is delivered to the kidney. So overall kidney function matures very quickly, and you'll see various values in the literature. But I would say between three and six months the kidney is considered to be sufficiently mature that is really not having a major impact on the distribution of chemicals at low levels.
I wanted to include a citation for several papers these are published a while ago in the late 90s and early 2000s to summarize what was known about TK variability in infants and children compared to adults and this quote is from one of the papers done by Andy Renwick where he says “infants and children do not generally represent a special group from a kinetic viewpoint” “and they would be covered adequately by a 3.16-fold factor applied to the mean data for adults.” He goes on to emphasize that a special consideration of children should focus on the potential for different exposures and higher sensitivity of developing organs compared with adults. I will also say that this does not specifically mention neonates. As you have heard and as you’ll continue to see, sometimes the information for neonates can be a little bit different than for older infants and children. So, while based on my review of the data today, for a general risk assessment for a low-level chemical in the environment, this statement is also still true for neonate. But in some situations, in pharmaceutical dosing, that is a population that needs the most attention.

Let me go back. This last point, and the folks [inaudible] talking about, the potential for different exposures and high sensitivity of developing organs compared to adults that really raises the question of the toxicodynamic. When we say toxicokinetics it is fairly well covered but what about toxicodynamics? This is difficult to study. One is what you do to the chemicals, this is what the chemical does to you. This is what will determine the response of a particular target organ for a given exposure. Compared to our knowledge based on toxicokinetics, clearly less is known about differences in toxicodynamics across the human population. What information we do have generally comes from a combination of developmental toxicity studies or multigenerational reproductive studies in rodents that include early life exposure.

So, the period of the most rapid development [inaudible] potential vulnerability from a developing standpoint is during fetal development such that if you have a rodent developmental study showing an effect on a developing organism that might be a real clue that that chemical has the potential to impact development that could persist even in early life post utero. If the data is lacking, options would be to use something like a SAR, structure activity relationship based, approach if you have a chemical that has structural similarity, or you could add additional uncertainty factors which I think Elaine had in her first talk.

The other information that we have that can help us address this question is on pharmaceuticals. One of the lessons in pharmaceuticals is very often inter-individual differences can be more important than life stage differences. My response is going to be less different than my response for somebody else's based on genetic differences. So as up toxicokinetics for toxicodynamics the main focus is in the first six months with a great and rate of maturation by highest. There are subsystems for which that difference can persist for much longer. If you think about skeleton for bone growth that is dramatic throughout childhood and a difference persists and neurodevelopment also is an area where development continues for much longer and is clearly the focus of a lot of research. That is also because of the potential for permanent adverse consequences to a developing organism. The best example is lead where an exposure to an adult would typically lead to effects that are reversible, any similar or lower exposure to an infant could end up with a permanent consequence. So those are the ones where I think the most focus is needed in terms of providing protection for early life exposures.

And the list of endpoints at the bottom are really the areas in which the greatest concern has been expressed. So, CNS or neurotoxicity and the potential for increased sensitivity that can continue for quite a while and Gary also mentioned lead, the potential for increased absorption from the G.I. tract compounds the potential for increased exposure already from a crawling infant that uses a lot of hand to mouth activity and already has higher exposure. Immunotoxicity
is also an area of research because there is still a fair amount that's not known about the
developmental immunotoxic. Endocrine-mediated effects and the skeleton and the bone which I
already mentioned.

So, what do we know from empirical data about the overall sensitivity of infants or children
compared to adults? The data sources we can consider are ones I've already mentioned
including the physiological differences that can contribute to toxicokinetics and studies that
involve exposures to young amateur animals most of the studies are done at high doses. Even
when we talked about the NOAEL that is generally still a high exposure when we think about it
in the context of human exposure which is generally orders of magnitude lower. I will provide a
couple case studies and situations where it's important to keep this difference in mind.

I also want to mention often these same data as both pharmaceutical literature and from rodent
studies that are cited when we raise concerns about infants being potentially more sensitive. It's
coming from this body of literature. So, in young infants the maturity of pathways as such they
can be saturated more easily than adults. We know that to be true. Gary provided an example of
that. Infants can be more susceptible to high doses to saturate those pathways. That has limited
relevance in many situations to lower exposures. And it's often been mentioned for
pharmaceuticals sometimes children actually require higher doses to achieve the same efficacy.

So, I want to look at just a few examples. This slide lists examples. We know infants and
children can have increased sensitivity and decreased sensitivity and both increased and
decreased. Or differential sensitivity based on dose. I will start with, I don't want to go through
all of these examples. They are described in detail in a paper we published last year.
Chloramphenicol is one that Gary mentioned for which we know that there's increased
sensitivity. There were administrations of Chloramphenicol to young infants, including premature
infants, given IV doses of Chloramphenicol as a wide spectrum antibiotic in the NICU with tragic
outcomes, and it was largely due to the decreased ability of these infants to metabolize and
excrete it. These were, the doses that these premature infants were given were extrapolated
from adult doses and there wasn't any adjustment done for that immaturity. So that is an
example where if you made an assumption based on adults you have absolutely an increased
sensitivity for young infants, certainly premature infants, and knowledge of the impaired ability to
excrete it would have been really important.

Propylene glycol I have under increased and decreased sensitivity and this can happen where
the infant has a lower rate of metabolism of something. So then, the question is, is the parent
compound more toxic or is the metabolite more toxic? In this case, the propylene glycol at high
doses, there can be acute CNS effects such that at high doses, a young infant could be more
susceptible to that endpoint but less susceptible to the [inaudible] that results from metabolism
of propylene glycol.

But the one I really want to mention is the case of differential sensitivity based on dose because
I actually think this is probably a fairly common scenario and we just don't have a lot of
information or case studies that really bring this to light. Both of the first two speakers have
mentioned Chlorpyrifos, so I actually have that one here. It's important for the increased
sensitivity in the young. The toxicity of Chlorpyrifos is mediated by its metabolite, the [inaudible],
which is the potent inhibitor of [inaudible]. Gary showed the data were rat pups dosed at doses
of one or ten milligrams per kilogram at postnatal day five versus postnatal day 12, there was an
increased sensitivity and at postnatal day 12 that increased sensitivity was only seen at the
higher dose, not at the lower dose. All of the animals had the same NOAEL, however. That
difference in sensitivity was seen at the higher doses, so the NOAEL for the rats was .5 milligrams per kilogram.

So there was work done and published a couple years ago by [inaudible] where they developed a life stage PBTK model for Chlorpyrifos and look at the changes at the rate of metabolism and found that in early life for doses of .6 mg and higher based on their life stage model in humans, they would expect that six-month-old infants that have higher levels in the blood so they would exhibit a greater response at .6 mg or higher. That is related to the fact that you have overruled the ability of the plasma to metabolize it. But at lower doses infants are actually predicted to have a slightly lower level of the Chlorpyrifos in the blood. Importantly, actual human exposure is several orders of magnitude lower than this. So, this goes back to what they are talking about with a NOAEL in the animal. But human exposures are sometimes lower. So, it's really important for us to keep in mind when we talk about data suggesting increased sensitivity, if there is data coming from studies where the doses of magnitude higher than what humans might experience, those findings are not necessarily relevant to the lower exposure level. One clarification for the Chlorpyrifos is we don't have \textit{in vivo} blood levels to confirm this. It's based on the pharmacokinetic model but there is good reason to believe that it is true based on the physiologically that goes into the model.

So where does this leave us with regard to the question of where the current methods are adequate? First without sounding redundant I want to say exposure, exposure, exposure to the extent we can really understand the potential for exposure is which will greatly reduce the uncertainty and allow us to better target the assessment for the life stage we are considering.

With regard to sensitivity, no single conclusion can be drawn regarding the relative sensitivity of infants and children compared to adults. I think it's important to recognize that this can change quickly and that increased sensitivity at high doses is not necessarily relevant to exposure with several orders of magnitude lower. The overall conclusion is the current risk assessment methods included the use of a default uncertainty factor including life stages is generally appropriate and protected for all age groups including infants. I will do a time check since I've already turned yellow. Just wave at me if I need to speed it up here.

So, I’ve been talking about [inaudible]-based approaches, using a tenfold uncertainty factor, how comfortable can we feel about that? What about the opportunity to refine assessments? I want to emphasize this is true both on the side of exposure and on the side of setting acceptable exposure limits, the hazardous site, if you will.

I will start with the case study on Phenoxyethanol where our focus was based on the hazard side. So Phenoxyethanol is a preservative that is widely used in consumer products. We have a rather large database and some studies are better than others. But we have repeat-dose tox studies in rats, mice, and rabbits. Some are dermal, oral, drinking water. They have different critical effects. There is also a significant PK database including oral rat data from blood, tissue, and urine. And for dermal rats, there’s also urine data. There’s also human data, there’s urine data following oral and dermal exposure and also urine data in premature infants following dermal exposure. It’s really a rather ripe data set to fit into a PBTK model.

When used as a preservative in consumer products, there are regulatory limits for the highest allowable level being 1%. So according to the method in the scientific committee of consumer science in Europe uses to define aggregate exposure to something like a preservative used across products the assumption going in is that your preservative is used in all products and is used to get the maximum level. So, it’s really important to keep that in mind that these numbers
make that assumption. Where I have the red arrow pointing is what the NOAEL is. Then we have adult exposures which assumes use of all these products on a daily basis and then down at the bottom you may see a value for infants and children, which is higher. That is where the original concerns were. Do we have a margin of safety if this product is used for infant products? PBTK modeling can be used to address a lot of areas of extrapolation and risk assessments. I’ve mentioned several here. For Phenoxylethanol all of these areas are just by the PBTK model including the bottom one which is life stage.

What we are trying to do on the left you see your rat with the does and you apply a PBTK model to calculate the internal dose. In this case, you’re looking at the area under the curve for both the parent and the metabolite. We were not sure whether it was the parents or the metabolite responsible for the toxicity, so we modeled both. On the right you have your human exposure with a human PBTK model. We have one for adults and infants to get a human internal dose. Then you can compare the internal doses and you have reduced uncertainty in the risk assessment. If you don't have these internal doses, it typically will have uncertainty factors for inter-species and would meet a margin of exposure of at least 100. If you can reduce the uncertainty with the kinetic handling of the compound by applying PBTK doses then you can accept a lower margin of exposure.

This work was published last year by my colleague John Troutman et al. working with others at the Dow Chemical Company and Jeff Fisher from NCTR.

I am not a modeler. I would just share the top lines of this. In the center of this slide is a rather typical schematic of what a PBTK model looks like. The boxes on the left showed the model inputs and the right is an example of what the model would predict. For the model itself you can see there are submodels for Phenoxylethanol, PhE, and its metabolite, [inaudible] PhAA, which are linked through metabolism at the liver. You can see in the red, we have input for exposure by the dermal route and by the oral route.

These slides, I just wanted to show a few that had the fit of the model once the data are input. I know the lines are fairly light. But you can see the left is a lower dose and the right is a higher dose. With both Phenoxylethanol and [inaudible] acid fitting the model quite well. If you look on the righthand side, it appears the model does not fit as well for Phenoxyacetic Acid and that is because there is inhibition of the excretion of it. At least, we think that’s the reason for it. You can adjust the models to make it fit better. The decision was made not to adjust the model because this is actually more conservative. We are predicting a lower [inaudible] for the NOAEL that would go into the risk assessment, so by not adjusting it, it actually introduces some conservatism.

This shows a bit of the data to human dermal exposure based on urinary excretion. You can see quite a good fit. This table summarizes key points from the modeling. On the left we have the applied doses with the rat NOAEL at the top. In rat I have the adult and infants below that. Because we had enough data to generate PBTK models now we can compare the internal doses for both the Phenoxylethanol and the Phenoxyacetic Acid and now compare the internal doses for both the adult and the infant. In all cases, an advocate margin of exposure is achieved. If we had more time, you would see that the biggest difference here is actually exposure, which goes back to points I’ve been making around the importance to understand that. That is a good example of how PBTK modeling can be used to reduce uncertainty going across species and routes and also high-dose to low-dose.
I want to mention a little bit about the exposure assessment because it's really important. The list of products here are all of the products considered in an aggregate risk assessment and the assumption is made that Phenoxyethanol was used in all products at 1%. I think you can quickly see it's probably not really what is happening. You can refine this as you can were data based on actual frequency and what is the level is it really 1%? And ultimately, do modeling of actual consumer exposure which in some cases can be validated with biomonitoring data available for this compound. Some work on this has been ongoing by a European task force that will likely be published in the next year showing the actual human exposures are at least an order of magnitude lower than what was estimated for this risk assessment.

Conclusions. Differential susceptibility of infants and children compared to adults can be a result of differences in exposure which is often more important and/or inherent toxicological sensitivity, which becomes more important when you are talking about pharmaceuticals with a low margin between safety and efficacy. Metabolic and excretory functions mature rapidly during the first six months. Most data suggesting potential increased toxicological susceptibility are from former literature or high-dose studies in rodents. I've shown you some examples there those have questionable relevance to lower exposures. In the end, the available data and analyses, including global regulatory guidance available today, supports the default tenfold intraspecies uncertainty factor as being adequate to protect infants and children.

There are some references that I referred to throughout the talk. Acknowledgments that I did not do any of the work for the PBTK model and here folks who did. That is it.

Rudman: We're running a little bit late, so we're going to leave questions for Dr. Felter for the panel discussion.

I like to now introduce Dr. April Neal Kluever, who is currently employed as a toxicologist for the Division of Food Contact Notifications in the Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, where she specializes in infant life stage-specific safety assessments. Dr. Neal Kluever has served on international expert scientific groups for the World Health Organization and the International Life Sciences Institute Health and Environmental Sciences Institute. Prior to joining FDA, her academic research focused on the developmental neurotoxicity of metals and pesticides. She received her PhD from Johns Hopkins University Bloomberg School of Medicine and has a board-certified in general toxicology through the American Board of Toxicology.

Toxicology Challenges in Lifestage-Specific Safety Assessments, April Neal-Kluever, US FDA, College Park, MD

Thank you for the introduction. I would like to thank the Society of Toxicology and the FDA for allowing me the opportunity to speak with you today on toxicology challenges in infants, or in this case, life stage-specific safety assessments. I've already forgotten I'm supposed to use this, I think. I don't have any financial or otherwise conflicts of interest to disclose. I would like to state the data and interpretations expressed in this presentation represent that of me, not necessarily that of the FDA.

In today's talk I will go through in little bit of background and definitions. I'm fortunate the preceding speakers have done a great job in providing background information, so I don't have to do that. I will discuss subpopulation assessment at CFSAN and also infants as a case example for life stage safety assessment.
Here we are at the US FDA Center for Food Safety and Applied Nutrition. Our mission is to protect and promote the public health by ensuring the food supply is safe, sanitary, wholesome, and honestly labeled. Our mission covers all domestic and imported food except meat, poultry, and frozen, dried, and liquid eggs. CFSAN focuses on safety at the population level. There may be situations to consider subpopulations.

By this point, you are probably aware that subpopulations are divisions of the general population on the basis of some distributing factor such as age, physiological state, or disease state. A life stage is defined as a temporal stage of life that has distinct anatomical, physiological, and behavioral or functional characteristics that may contribute to different susceptibilities to chemical exposures compared to the adult population. I have taken this definition from the work of Susan Makris’s work in 2008, which is a very good work to read in full.

But how are subpopulations connected to life stages? EPA organizes this has included consideration of age groups or life stages. This is essentially life stages are a type of subpopulation in which the distinction is generally age or stage in life.

That being said, what about subpopulation assessment at CFSAN? They held a Food Advisory Committee, or FAC, meeting in which we requested advice from the FAC on how to integrate scientific consideration for several populations into our risk assessment procedures and methodology. The proceedings are published online at the links provided here. I highly encourage those interested to review the materials online. There is video recordings and transcripts. Just so people perhaps who are unaware of how the FAC works is composed of external experts and skills relevant to the areas and CFSAN will pose questions to the FAC to solicit guidance. I did not actually progress. In this case, we asked the Food Advisory Committee specific questions on how to consider subpopulations. There is actually quite a bit to the report. But I am summarizing it here. There were three scenarios that the FAC described in which CFSAN may consider a separate risk or safety assessment. The first scenario they listed is when there is a clearly bi- or multi-modal distribution to the functioning of an influential enzyme or system such that the expected risk will be distinct from the index population, generally the adults.

I want to pause to emphasize this is a high bar in terms of data. You have to have quite a bit of data associated with a chemical to determine if your chemical meets the criteria. And that may be the case for some widely studied environmental contaminants. We don’t always have this level of data, but there are two other scenarios.

The second scenario is when a life stage appears vulnerable based upon critical windows of toxicokinetic immaturity or toxicodynamic sensitivity and this sensitivity has been characterized in dose response studies. The third scenario is when a subgroup or life stage may receive a disproportionately high exposure to a food, product, or environmental media that contains toxicants that are of particular concern to the subgroup or life stage.

So that is the FAC recommendation. Now, I am going to go into the specific case of infant safety assessment. Infant specific safety assessments which is an example of life stage specific assessment are performed for food contact materials intended for use with breastmilk or infant formula. I want to also acknowledge that in life stage specific safety or risk assessment they also be performed in CFSAN outside of food materials if the need arises but since my area of work is with food contact materials, I chose to use this as the example.
Now what do we mean by food contact materials? In the most simplest sense, packaging. What is in infant specific food contact material? Again, something that contacts breastmilk or infant formula. A bottle, the nipple, the adhesive used in the nipple or the bottle. There is infant formula cans, there is coating in the infant formula can. There is also a common misperception, is that breast-fed infants may not have food contact exposure but that is not the case. Breastmilk is stored in storage bags. It is delivered to the infant in bottles which use nipples. Both breast-fed and infant formula fed infants experience food contact material exposure.

So again, someone else's is timing my slide progression, sorry about that. These are some examples of infant food contact material. Now infants experience a different exposure scenario than other life stages. They have an increased food consumption compared to other age groups it is on a milligram per kilogram body weight per day. I want to emphasize here another misconception I commonly encountered is that people generally assumed that the adolescent male had the highest intake per day. That is actually not the case. Infant as an age group have the highest food intake per kilogram body weight, not adolescence. They also consume a restricted variety of food products. They may consume a sole source of nutrition for up to six months, that is infant formula or breast milk and they do not exhibit the same consumer patterns as other age groups. If you think about an infant, they are consuming one or two food sources delivered through bottle with the nipple etc. There are also, to a large extent, restrictions in terms of the brand and the items that are delivered. Parents don't always stick to one formula brand, for example, or if a particular bottle is well received by the infant, that is the bottle and brand that will be used. So, in contrast to older children, in contrast to adults, these older age groups will consume a variety of food products, packaged in a variety of materials. That is not the case for infants.

To go back and say because of the restriction in both people type and the consumer patterns, there may be a potential for a higher exposure in infants in one particular migrant from the packaging compared to a scenario for older age groups.

And I would like to say that this satisfied the third set of scenarios that the FAC provided. That infants they receive a disproportionately high exposure to a food product or environmental media.

Now regarding infant biology, by preceding presenter has set the stage quite well here in demonstrating cases where infants are experiencing very rapid changes both in terms of developing physiology, that is the processes of absorption, distribution, metabolism or excretion and in developing systems. Now, we recognize that infants as a whole, their entire body is undergoing rapid changes and proliferation with the different organ systems, but I have highlighted here several systems that we consider a particular interest for the infant life stage due to the potential in these systems for delayed or latent effects or potentially permanent adverse effects, and these systems are the reproductive, endocrine, neurological, skeletal and immunological systems. I have provided two citations below that will help people interested in reading in greater detail about these concepts.

So, in terms of developing physiology or pharmacokinetics, I don't need to go into this too much because it is already presented, but essentially infants exhibit different absorption, distribution, metabolism and excretion and storage of some chemicals compared to other life stages. There rapid changes in the first six months, and actually, in the neonatal period, the first four weeks after birth, there are changes on a daily timescale. Altered PK/TK parameters can have a large impact on toxicity of a chemical and infant susceptibility, as has elegantly been described previously by, sorry, Dr. Ginsberg. I have stage anxiety reading names. So, I apologize.
Here are two more examples. These are big chemical classes. That I just used to re-illustrate this point briefly. Here are two chemical classes that are well known and well studied, dioxins and methylated xanthines. In infants, dioxins have a faster elimination primarily determined by lower body fat which is present in infants. And methylated xanthines have a slower elimination largely due to deficient CYP1A2 activity in infants compared to older children and adults. Of course, this is only one facet of the story of the toxicokinetic and dynamic profiles, but here are two examples, one in which the elimination is faster, and in one in which the elimination is slower.

Okay talking about pharmacodynamics, this is about target as that has already been described. Infants, they express more or less of the biological targets of a chemical than other life stages. There are some relatively easy examples of this. Receptor ontogeny is one of those. Neurotransmitters change very quickly. The expression of type and subtypes of receptors change rapidly during development. One can anticipate that antagonist of these receptors may have a different effect in juvenile stages than in adults.

Another example is infants exhibit a high cellular turnover globally, that is, throughout the body. This may provide increased to targets for carcinogens. Of course, all of this again is just one facet of the picture. Increased target of carcinogens would be dependent on the fact that the infants are metabolizing chemicals to the ultimate carcinogenic species.

So, I have the hope that I have demonstrated that infants demonstrate at the minimum two of the three criteria and I'm saying that is a minimum because they may actually be scenarios and there are scenarios in which we have the data sufficient to satisfy criteria one, but not all the time.

So, what are the steps in infant life stage assessment? The first step is the estimation of the infant specific exposure to the substance of interest. This includes consideration of infant specific factors such as weight, intake, and consumer habits. And I appreciate that Dr. Felter spent a lot of time on how important exposure is. If there is no exposure, as a toxicologist, I don't have to do anything. There is no exposure. But I need to know what the exposure is when there is exposure for me to place that infant contact with a safety assessment which is a second set of steps.

The remainder of my talk will be focused on a safety assessment. That is what we will move into. So, from a very high-level viewpoint the challenges in infant safety assessment are not unique to any type of assessment pick the basic questions are when to test, what to test, how to test, and how to interpret the data. Everyone is familiar with these questions. However, what is different is there are specific caveats to consider what we are talking about the infant life stage.

So, with the first challenge days when to test? The FAC provided guidance regarding when a separate safety assessment should be performed. So, we know we should be performing a separate safety assessment if we meet the criteria. However, it remains an expert decision to determine what additional or specialized toxicological testing is needed to support if and safety. What I mean by this? Well generally in my office if I receive a new petition or submission for a food additive, it will come with the data associated to demonstrate the safety for the general population. It is the toxicologist job if they were viewing and infant product to determine if that information that covers the general population is sufficient to cover any potential safety concerns specific for infants. And this is not a straightforward question to answer.
But what are some ways that we can identify or prioritize chemicals for candidates for specialized testing? What we can harness the wealth of information that is available in public literature that includes peer-reviewed journals and other government reports. There is screening batteries such as the Tox21 program. Other screening assays that are being developed. There are in silico prediction approaches, QSAR with bench, and this is a mathematical approach to compare structural elements of a chemical in terms of physical chemistry, there we go of the structure. And compare it to known toxicological properties of other chemicals to determine whether or not there may be a potential hazard and then there are other hazard identification tools such as the approaches like the adverse pathway approach that looks that chemicals broadly looking for similar types of objects across chemical processes. These types of approaches may determine when a chemical is a candidate for additional specialized testing to support infant safety.

Now that you have a chemical in mind that may need specialized testing the next question is, what has to be done? This is an extremely challenging question. There are a variety of developmental and reproductive toxicology tests. Dr. Faustman provided a schematic that showed testing and which life stages are covered under which tests, but we can start with what I consider the elephant gun of DART testing, which is the generational developmental and reproductive testing paradigm. This is your extended one generation study. Your one generation study, your two generation studies, that exposed animals throughout all life stages, they capture a wide variety of reproductive developmental and systemic endpoints. They are very costly, and they are very long, and they take a while to complete and to analyze, but they are comprehensive.

Then there are more targeted and smaller study such as the prenatal toxicity testing, also known as the segment two study or the teratology study. There is a perinatal/postnatal toxicity study, which encompasses the late gestational period through lactation, and again this would be equivalent to segment three study. Then there are additional other specialized juvenile animal test protocols that will vary in the exposure window the endpoints that are measured. Trying to decide which one of these studies may be best suited to characterize an infant safety concern is not a straightforward question.

Ultimately the question becomes should a large study that captures many endpoints such as the generational study be recommended to support infant safety, or should a smaller study be recommended? To answer that very difficult question we have initiated two research products. The first of which I call the Gen-DART project. This the assessment of the utility of generalization developmental and reproductive toxicity testing for infant safety assessment. This is ongoing regulatory research that we initialize in September 2014. So far, we have completed the analysis of 40 Gen-DART studies on 37 food additives. We have expanded our study library by harnessing data from external sources. This is so we can expand our sampling of the chemicals space.

Things that we are looking for, they have to do with what data specifically do these studies provide that will be useful for infant safety? How often do juveniles exhibit different sensitivities than the adults? How often can the adults predict the toxicology profile in the juvenile? These types of questions are the questions we are seeking to answer.

Another arm of research that is parallel and related is what I call the JAS. It is the assessment of the utility of juvenile animal study protocols for infant safety assessment. These are studies that are specific type of protocol that are recommended routinely at the Center for Drug Evaluation and Research to support nonclinical safety of drugs intended for pediatric indications. This type
of protocol is very targeted. It is almost a mechanistic study in which it is the age of the animal under study is highly targeted. The endpoints are highly targeted. A lot of mechanistic information is known generally going into the study. The flip side of the coin to the developmental generational study in which a broad range of inputs are captured over an extended time. In this case, a fewer number of endpoints are captured over a shorter and targeted time and we are interested to know if this has a role in infant safety assessment. We have formed a collaboration with our colleagues. We initiated research into looking at the studies and we have a pilot capture underway associated with 60 drugs.

Now because both of these projects are not complete, I cannot share any of the data for you but I do recommend, if this is of interest to you, be vigilant because we do hope in the effort of transparency to share through peer-reviewed publications what we can as long as we can maintain our confidentiality requirements with proprietary data.

So, moving forward, these regulatory research projects will provide scientific framework for the recommendation of specific study protocols to support infant safety. And we hope that this research may also informed question on the interpretation of study data in the extrapolation of potential hazards from the test species to human infants.

What is another challenge? Well, how do we interpret the data track now that we've done the study, what do we do the data? How do we use that data to extrapolate the human health risk? There are some challenges here, some challenges to the challenge if you will. The first is that nearly all DART study designs have several major areas of uncertainty. The first is regarding test article exposure pick.

This is a schematic from the OECD test guideline, the extended one generation study. You can go to their website to read the entire protocol ever this schematic is interesting to show the point which I want to share which is if you look at the yellow line, that is the ghosting for the exposure paradigm. You can see that in this study animals were exposed predating, during mating, during pregnancy, throughout lactation, and the offspring are exposed directly to the test article whether that be through diet or water or world from the post weaning parried and onward.

Now it is important to emphasize that prior to weaning the pups are assumed to be exposed through the maternal unit whether or not that is a simple transfer or through lactation. You can add a toxicokinetic cohort to this study. That would greatly increase our understanding of what the exposure is to the pups but generally, toxicokinetic are not included so we generally do not know to the extent pups are exposed to the test article if at all.

How do we addresses uncertainty? Well we can recommend direct dosing up. In DART. That would greatly increase our understanding of how much the pups were exposed to the test article. We can also recommend toxicokinetic profiling of chemicals to be tested in DART studies or the addition of the pharmacokinetic cohort.

Finally, there is physiologically based pharmacokinetic modeling as audit by the previous speakers however I would like to emphasize as they did that these models are again only as good as the data that goes in and you need to have [inaudible] model to be sufficiently developed to provide day to provide confidence in the predictions that they provide.

What is another major area of uncertainty? There is a lot of uncertainty regarding test model selection. All currently validated regulatory testing protocols use mice, rats, or rabbits to test for developmental effects and what we are talking about effects in the early postnatal time period,
we are almost exclusively talking about rat pups because those are the predominant species of choice for postnatal toxicity studies.

Well, rats have a different timeline than humans. In every organ system. Here is one example, here is a kidney. This is a timing of completion of nephrogenesis for various species. Man, or human, as you can see the nephrogenesis completion is completed prenatally and the rat is quite delayed. They are well into adolescence before the nephrogenesis is complete. You can see the other animals listed tend to vary to some degree in the timing. This again brings up the point that every test conducted, every chemical under study should be considered on a case-by-case approach, what the target organ is if you know the target organ, what is the target organ timeline in the animal species of your study, what is the timeline in your human, and how can you interpret the animal study in the context of human development? That is not easy to do.

How can we reduce uncertainty and assist our ability to extrapolate this information? Well, we can look at human data. I'm just going to say it straight out. There is not a lot for environmental chemicals in human children there is not a lot and there is not going to be a lot for very good reasons. There is some pharmaceutical data. As Dr. Ginsberg show you can use to inform the space environmental chemicals because drugs are processed through enzymes in which environmental chemicals are also process. So, you can understand a little bit of our environmental chemicals in the pharmaceutical data. You can also use again PBPK modeling when the models have sufficient data to provide adequate predictions. And then there is development or validation of additional models or test systems. For example, there are pieces in which in vitro test systems have helped us to understand better, how the human differs from the animal under study. These can be included in PBPK modeling to help us understand potential differences.

The mini pig is an animal model that models the human gastrointestinal moderation quite well, however the problem with the mini pig is that they have not been sufficiently validated in as many sufficient and points as the rat example. There are efforts underway to develop for the mini pig, but that battery has not received international validation, so neurotoxicity is a significant area of investigation for developmental exposures and it is not yet at this point adequately characterized in the mini pig. But as time progresses, we have more alternative models that may be sufficiently validated for use.

In summary, I hope I demonstrated that infants fulfill the CFSAN Food Advisory Committee advisory criteria as a life stage of interest. Infant specific exposure and safety assessments can be performed if relevant data are available. And infant life stage specific assessment is an emerging field of regulatory research. There are regulatory research efforts underway that will optimize the approach to infant safety assessment. And that is that.

I have left some time for questions and then we have a panel discussion.

**Rudman:** April, you did a great job. Do we have any questions for April? Please come to the mic if you do. No questions. We will proceed to the panel discussion led by Dr. Goering. Will the speakers please come up?

**Roundtable Discussion, Peter Goering, Moderator, and all speakers**
Goering: I would like the audience to stay. If you would like to take 30 seconds to stand and stretch, you can do that. I think we will look at trying to have 30 minutes of discussion here, and we will gather at the front here.

Thank you everyone for your attendance today. Thanks to the speakers for some wonderful talks. And just highlighting the complexity of this issue. It is a really, thanks. And I wanted to let the audience know that, yes, you have been silent and we ask you to hold your questions, so please feel free during this session to come to the microphone if you are unable to do the stairs well, just raise your hand and we will have somebody bring you the microphone, but we do want to invite a lot of audience participation. I also have a computer, so I want to invite our online participants to email their questions on the chat function, and I will try to monitor those as well as the discussion here.

And Elaine, I just want to make sure if you are still on the line. Maybe you are on mute. If you could just interrupt me when you come online, I would like to know if you are there or not. Speakers, I would like to invite you, I will try to throw questions out, but please feel free.

Faustman: Hello. Peter, I think I was on mute and it was not me. Can you hear me now?

Goering: Can the audience hear her? Because it is not very audible of front here. The audience is pointing more volume for the speaker on the phone.

Faustman: Is that better?

Goering: No. It is not much better. Just a minute, Elaine.

Faustman: Okay. I don't think that was a meditative hum. It was more of an auditory feedback. Can you hear me now?

Goering: Yes, we can.

Faustman: Again. I am so sorry to not be able to be there in person. I was actually really excited to hear even though I have seen the presentations ahead of time. In the delivery it is so much more fun to hear this. I wanted to ask, I noticed that even though I said that there was a role for in vitro information for informing some of these processes, I noticed that everyone else deliberately did not say anything about that. Could we tackle that one first because I think I heard a real call for additional data, how do you see that maybe fitting into this overall portfolio of ways to inform ways we do safety assessments? Thanks.

Ginsberg: On the toxicokinetic side, there are liver bank samples for in utero and postnatal age windows as well as for adults. And so, it would not be a major research effort to run your chemical of the week, whether it is coming from packaging or it’s a food additive or something else, to these ontogeny-based liver bank specimens. We could do a lot more with the liver banks than just see what the CYP level is, but we can actually look at rates of chemical turnover, and so I think that that’s very underutilized resource at this point.

Faustman: Anyone else want to say anything, like April?

Neal-Kluever: I can contribute. Right now, the integration of the in vitro high-throughput screening or alternative models has not been fully integrated into the safety risk assessment. There is still sufficient validation that needs to be performed for us to really integrate it at higher
levels of safety risk assessment. The role that I see for these types of method is performing hazard identification for example. They may provide information that is in the context of everything known about that chemical, may indicate whether there is potential for altered susceptibility ability across life stages. Again, for example, interacting with particular targets that is expressed at higher levels or lower levels, we can place that into particular context for the safety assessments, but we would not necessarily be facing a regulatory decision on that type of information, but it would feed into it in a supplemental or hazard identification manner.

Faustman: Excellent. Thank you.

Goering: Elaine, how about you? Do you have some opinions on that?

Faustman: It was interesting to hear Gary say he had some ideas. I actually think there is some window of opportunity and I think we just need to be a bit more creative and that is why I think it is so important that we have this framing of the information that is going in and so I actually think that there is interesting models that are addressing metabolism differences that we use but we kind of count as mechanistic data not necessarily as in vitro data, and this challenge that April particularly laid out there that talked about the appropriate animal models to use, I think there are some ways to look at how different are the rats, mice, and mini pigs in the in vitro systems, how much of that difference in timing can we develop so developed life stage specific in vitro assessments, for example. So, I actually think we just need to be a bit more creative in how we pull some of this information forward. But I think it could help answer and there are recent efforts going on from [inaudible] to look at systematic review to pull in more mechanistic information into those assessments so that it does not say on the cutting room floor so to speak during systematic reviews. I have hope that we can make some differences on that.

Goering: Thanks, Elaine. I have a couple questions from online and I think they were good questions for after each of your talks. They could engender some broader discussions here. The first one is for Susan. Our online participant is asking you to explain what you meant by Chlorpyrifos in the young at low doses resulting in lower [inaudible] levels due to liver metabolisms?

Felter: So, I was referring to a paper, I believe it was published in 2014, that [inaudible] worked together to develop a life stage [inaudible] model and were looking at the influence of dose on the level of the Chlorpyrifos [inaudible] in the blood. There were some competing things happening, that at higher doses, the ability for an infant to detoxify the level of [inaudible] in the blood is limited. There is a saturation of that. At high doses, you see that they are more sensitive and that is what was shown in the rat study. However, [inaudible] doses where you have not saturated that capacity the larger liver size of an infant will actually lead to lower levels of the [inaudible] in the blood. This becomes really important we are extrapolating from high-dose animal studies or even high-dose pharmaceutical exposures where what we see at high doses that may have exceeded a metabolic or excretory capacity of an infant, as long as the exposure is lower than that, then that saturation, if you will, no longer becomes important in the risk assessment. So, the paper that I was referring to actually found sort of a cut point, if you will, of 0.6 milligram per kilogram, which was right around the level of the NOEL in the animals, that at exposures higher than that, you would expect human infants to be more sensitive, and we also saw that the rat pups were also more sensitive.

Based on the physiology and development of human infants at lower exposures, and we don't have data of rats at these lower exposures, well we have no effect levels anyway, but you would expect the human infant to actually have lower levels of the [inaudible] in the blood. I don't think
the levels were a lot different. I think was relatively a small difference but at low levels you don't see the higher level that you are, when you are up in the level of the NOEL in the animal study, an actual human exposures are orders of magnitude lower. So, it really highlights the point that we are becoming much more aware of in toxicology. That is the influence of dose. It is relevant for exposure in adults as well that when you have exposures that are orders of magnitude higher than we are actually exposed to, we have to be careful in interpreting those data if we are dosing animals, for example, at levels that have saturated the ability to metabolize or excrete the chemical, and that was just a great example of how that plays into life stage.

Goering: Thank you. We have a lot of questions coming in online, so I am going to try to get to them. April, I think the number of people were very interested in the, I'm going to say next-gen, but the one-gen studies. They are asking you to add to your discussion of the assessment of the one-gen program. And specifically, is asking, will you add increased kinetic assessments?

Neal-Kluever: Well I am not quite sure how to answer it, but I will answer what I think the question is. For the one, begin with, the assessment is an assessment of a variety of generational protocols. We have one Gen. We have two Gens and we even take in more relative data from a continuous breeding protocol in which we take just the first litter of data or data from the first litter, so things that give us a handle on postnatal, early postnatal exposure and exposure in the adult generation as well. So that we have an adult and juvenile cohort to compare. We are not performing additional studies, these are a retrospective analysis of studies that have already been performed. We are harnessing the data that we already have. Moving forward I would like to see, from a scientific standpoint in my own interest, more toxicokinetic data because I have learned to highly valued the additional information that you can learn about what is going on, on multiple different levels, [inaudible] levels, organ tissue levels, there is a wealth of information that can be provided with relatively little investment when you are talking about an already expensive study, adding a cohort may not be a huge additional burden or as I mentioned range finding or standalone toxicokinetic studies. That would be something scientifically I believe would add a lot of additional information to help us understand juvenile versus adult differences in susceptibility or sensitivity to chemical exposure.

Ginsberg: So, from the perspective of adding to the scientific body of evidence on postnatal or juvenile or infant sensitivity, I think that the approach, and I'm not sure exactly what you're talking about when you talk about postnatal [inaudible] dosing for example, but if that is part of a whole life study or a whole reproductive cycle study and you are not quite sure where the sensitivity is coming from in your output outcomes, it may be great from a safety assessment where you could say, well, we have specifically dosed every phase that may have a sensitivity for renal development or whatever development, and so, we could say for that chemical for that case, we know what the safety or lack thereof is. But, and the FDA Redbook protocol for whole life [inaudible] testing is like that. You get one outcome for that chemical and it is a whole life protocol, but we do not know where any extra vulnerabilities are coming from, so to make predictions for other chemicals that are not going through that, we don't know specifically what is the postnatal specifically versus the in utero sensitivity and whether it’s a [inaudible] or dynamic thing. So, I am just saying it would be great from a toxicology body of evidence if there is some separation out, so we can identify vulnerable windows as much as we can.

Goering: Response?

Neal-Kluever: Response? Yes. And I totally agree. 100%. In some cases. It is very important to try to get a handle on where the window of susceptibility is. When we're speaking about infant products there are products that are exclusively used by infants. They are not used by other age
groups. So, when we are reviewing the safety, you want to know the infant specific hazard and currently, developmental and reproductive toxicology programs, test strategies, don’t target the early postnatal time periods exclusively. There is almost always an *in utero* or later window involved as well that you don’t have a discrimination between stages. It can be very challenging to determine is this, for example, an early life states specific risk or is this an *in utero* risk? We cannot differentiate. We tend to take the conservative approach and lump them together because we do not have the data to differentiate in the majority of the cases.

**Ginsberg:** It is also not necessarily conservative to not specifically dose postnatally because *in utero*, you have the [inaudible] system which is completely changing delivery and postnatal, now, it’s going directly per body weight, you might be getting a completely different dose to the postnatal, so I think having a segregated postnatal exposure window would be helpful as we have learned from early life cancer data.

**Goering:** We’ll use our best judgment on that. Allen, you can raise your hand if we have crossed the line. It is great that audience members are at the microphones. Do any of you, either of you, have any response to this discussion that is going on? OR a new question? Do you have a follow-up? Tom [inaudible] next.

**Audience Question:** Thank you all. This has been very interesting. I think one of my concerns is that in what I have seen so far developmental studies, they are looking at endpoints that are time constrained for a very short period to time after birth. And for what we have been hearing, there are long-term consequences of some of the early exposures. And we, one of the deficiencies I see in the system so far is that there is no long-term and a good selection of some endpoints that would help us understand how those early exposures are affecting chronic diseases or increased the risk for some diseases later in life. One of the main points that Elaine made today, and I think April alluded to it, too, is the latency period. For some of the developmental studies we see postnatal assessments but there is very little on what happens, you know, six months later in the rat, or if you [inaudible] to humans many years later.

And the second one is, Susan mentioned in her example of the propylene glycols, that there were two different sensitivities. I wonder what is importance of the selection of endpoints to identify those sensitivities? How we make some advancement in the science of how we select endpoints to improve predictions of how humans will react to exposures [inaudible]?

**Goering:** Susan, do want?

**Felter:** I will work backwards because there is a lot of questions in there. With regard to the propylene glycol example, where you can have increased sensitivity for one endpoint and less for the other, typically in risk assessment we are going to want to protect the entire human population and all life stages against all endpoints, right? So, the question sometimes is whether we are comfortable that we understand all of the endpoints, and with regard to this symposium are there potentials for toxicity, either something you don’t see in adults at all would be of concern in early life, or whether they have increased sensitivity. So, the point of that as a case study was not necessarily to say you would do one or the other, you would want to look at all endpoints, right? And recognizing that just because you see increased sensitivity for one endpoint does not mean you will see it for all endpoints.

**Audience Member:** Right.

**Goering:** One more question.
**Audience Member:** Just a follow-up, for instance for neurological endpoints there are data that April may be analyzing on her gen DART project. You won't have in the dog population. You will only see it in the pups who were exposed either postnatally or in utero and postnatally. But then those pups, it is important to understand or to figure out which are the endpoints that will be looking at, at the neurological level. And follow them through life. I think that is a gap that we are missing.

**Faustman:** So, this is Elaine Faustman. I think you have raised an extraordinarily important point.

**Neal-Kluever:** I do believe, in my scientific opinion, one of the strengths of the generational protocol is the ability to test out to the reproductive function, for example, the neurodevelopment and the adult neurological capacity of the animals exposed early in life. Now are all latent endpoints or possible latent effects tested in a generational study? No. I don't think there is any study design that can currently do that. But, if you have concerns for particular endpoints, there are studies that allow modifications to the protocol to incorporate additional endpoints. So, there is flexibility of study design, but it is always a challenge to sufficiently, adequately address the potential for latent or delayed effects. It is a general challenge for developmental toxicology.

**Goering:** I wanted to remind people that there is a lunch with the speakers following this session, so if you have additional follow-up questions that will be a very informal place. We are having lunch? We are not having lunch? Yes. Good. That’s most everybody.

**Audience Member:** A question for clarification. You started talking about the window of susceptibility in the developing organism and there’s some time frames that they’re most susceptible. It sounded as though, Gary, Dr. Ginsberg, was sort of saying it would be of value to have that information developed, but when the window of susceptibility is maximum? Prenatal day something or other, 12, 14. Are you referring to the development of a regulatory experimental data to answer that question? It seems like the window of susceptibility when you are talking about a food additive, food chemical, is not, I don't mean this exactly, is not relevant when you are making a regulatory decision. Because you’re going to be giving exposure throughout the time period of development. For pharmaceutical, it is relevant because you may be targeting the use of a pharmaceutical for a particular time period. But for a food related chemical, that question does not seem to be relevant to make a specific effort to define that. You can do that as an experimental background information [inaudible], but not as a part of a regulatory database that you would want to put together.

**Ginsberg:** I understand that. And I understand that the testing may be driven by a regulatory need to understand the safety throughout the lifespan, including all those early life developmental windows. But I am also saying that if there was a way to separately test some of these windows, we will know, number one, when for example the brain or the kidney in a rat, your test system, is maximally sensitive so that you would make sure that you are covering that for both, for example, we may not have that data historically. We may for some previous chemical that has not gone through it, we may see that that factor is fivefold or tenfold more than the adults. Or maybe it is really important to go back and test these things. From an environmental perspective, a lot of the chemicals we see have not gone through developmental neurotoxicity, developmental immunotoxicity, and so if we are starting to learn from the pharmaceutical literature what those postnatal vulnerabilities are, we may learn a lot on the environmental side of things that we are not aware of yet.
Goering: Elaine, I assume you are still there.

Faustman: Yes. Hello. Can you hear me? Thank you for your patience in waiting, but go ahead.

Audience Question: Susan, I had a question about exposure. When you are talking about exposure, do you consider both leave on and wash off in the personal care products for the infants, especially with the dermal? And then for April, I had one question, for the developmental toxicity, do you consider the zebrafish model in your evaluations at all and how do you?

Felter: Yes. Your first question about the infant exposure, absolutely. There are both leave on and rinse off products for adults and infants. There is categories for each. There is clearly more categories for adults than there are for infants. I actually have two separate rows for infants, and one was representing sort of the general types of products that would include cleansers, lotions, and things like that, and the second row was more related to the nappy area, which is the European term for the diaper area, products are used specifically in the diaper area. And those two were added together for infants. It did include both leave on and rinse off and the way the exposure is reflected is only a portion, so if you applied X amount of the rinse off product, only a portion of that would stay on the skin because you wash it off right away. So, those are both accounted for.

Neal-Kluever: About the zebrafish, personally, with my background in developmental neurotoxicology, I see a lot of appeal to the zebrafish model. I am especially interested in the behavioral batteries that they are developing with the zebrafish. There are caveats, including exposure caveats. They are in the water. [Inaudible] exposure to the test article. The route is different. But there are caveat to the zebrafish model. But I do think the model shows a lot of promise. How it may be integrated is a bigger question, but again, not to sound like a broken record, I do believe that the zebrafish may provide hazard identification or supplemental information that can be used in a broader context of a risk or safety assessment.

Goering: Good. Question?

Audience Question: There was a lot of discussion today about interchild variability or variability in general when we talk about epigenetics. As humans, culturally, socioeconomically, that can impact your exposure, but a lot of our modeling is using rodents that are, that are basically the same type, there's no variability there. So, when you think about that, what do you think about that? And also, for Dr. Ginsberg, when you think about, when you look at differences in interchild variability, where do you think that comes from because when you look at your liver data, for example, do you see variability in enzyme profiling, for example? Just across cultures? And how do you all feel we should address this? Should we be mixing different mice breeds, for example, in our studies?

Ginsberg: Well, in the genetics area, the answer is pretty simple. And that is that a lot of the data we have is from humans that are exposed to pharmaceuticals. We have some information on variability because we have sometimes 10, sometimes 20, sometimes only three or four children of a certain age window and reorganize that in the database that I described. So, the human data, number one, we don't have to worry about the extrapolation from rats or mice. But it is of course still limited by the fact that they are all usually not well. We try to pick children whose tissues, whose liver was intact, they didn't have some liver disease in the database, otherwise we didn't include them. But there are sick kids, usually, that are getting tested for their pharmacokinetics, and in the liver bank study again these are cadavers. So, something happened. But there is a fair bit of variability in the data. And we make use of that to express,
not only the differences in the central tendency, but the percentiles back to the adult mean, which is an important consideration.

**Faustman:** This is Elaine, I would like to respond to that.

**Goering:** If you could hold on just a minute. I think since the last question brought up different factors that might play a role in susceptibility exposure, toxicokinetics and toxicodynamic differences among individuals, I had this question last evening when I was looking at material, and I see it came up online. What is the panel’s view regarding the microbiome and safety assessment? This individual says that there are known microbiome changes with age with individuals. [Inaudible] 101 factors that change microbiome.

**Audience Question:** In the closed captioning, Dr. Faustman has been responding, but she hasn’t been being heard in the auditorium. So, there are like these little comments saying, like, “This is Elaine. I would like to respond to that,” or “Hello, can you hear me?”

Goering: Wow, that’s too bad.

**Audience Member:** So, I’m so sorry, I didn’t want to interrupt you.

**Goering:** I didn’t see that on my computer. If Elaine is there?

**Faustman:** Yes.

**Goering:** We would like to open that for her while the rest of the panels think about the microbiome.

**Faustman:** Can you hear me?

**Goering:** Yes.

**Faustman:** Great. Thank you. Keep considering the microbiome. But the point that I had to this last one about variability across cultural communities and things, this becomes very important and this is important at several levels. Both from the animal and also from the human, so let’s take a human situation first. As you know, many of us are a very involved in the [inaudible] studies and looking at the reflective polymorphism that are coming out and sources of variability at the genetic level from the [inaudible] genomes project. We work with Hispanic populations and we were very interested in Latino differences in metabolism. We’ve been looking at how much additional variability one needs to include in pharmacokinetic models for things like pesticides when you have a population that is primarily of one cultural. And I can say that there is developing databases that allow one to start to look at that, so you can [inaudible] your populations of interest or use or concern to be able to answer those questions. Stay tuned on that. And I will give a plug for FDA for the drug literature. I don't know if anybody has gone in recently to look at their [inaudible] databases and the relationship to specific drugs and products, but it’s one of the best annotated datasets around. That is just a call out for resources that we have not discussed so much. It is less usable for the broader portfolio [inaudible] environmental and food residues and things that are out there, but it certainly is extraordinarily well documented, and it pulls you in in a good way.

The second thing I wanted to mention is going back to some of the *in vitro* models. As you know there is large efforts looking at collaborative cross models, so that the mouse strains that we
look at have been linked then back to actually specific genes on the chromosome and that could be mapped back to human diversity. Using those particular animal models, let's say, to answer question in immunotoxicity, allows you to then link back directly to pathways of interest and variability in human populations. So, watch that. And even more, I am going to give a call out to Ivan Rusyn, who as you remember, who was one of the organizers of these sessions. But they’ve been using [inaudible] cells, so these are cells that you can put into culture from individuals and they reflect the human portfolio of polymorphisms and variability, so that is fantastic to be able to pull that in, and I think that is one of my points about the excitement of pulling in in vitro and mechanistic data into some of these areas where we don't have full-blown in vivo studies. So, that was one thing I wanted to mention. Now back to the microbiome.

Goering: Elaine, I don’t see any takers right here now. So, if you could chime in on that we would appreciate it.

Faustman: Sure. No, I think this is extraordinarily interesting. We have been actually doing studies on looking at the oral microbiome and the effects of different chemical exposures in the portfolios bacteria that are present there, and what that might mean for disease state. That data is not all published yet, but it’s resulted in us going back to the literature. I have not seen one [inaudible] for all the chemicals and chemical crosses that can impact the portfolio of chemicals in the microbiome, but it is big, and it should have huge ramifications for what is in store, what forms are absorbed and also a variety of other factors from G.I. absorption. But remember, the food literature and breast milk literature is one of the places where the earliest microbiome assessments were done and it is very interesting, there's great literature out there that starts to talk about when the child microbiome is formed compared to the parent, the mother’s microbiome, and it follows and tracks with this early introduction, of course, other foodstuff. What is interesting about that in particular at least from my standpoint, is how stable or resilient those populations are versus non-resilient [inaudible]. I think that is emerging, but certainly it is of great interest.

Ginsberg: I am not sure how great the evidence base is for this, but apparently there is a clinical connection between peanut allergy and normal vaginal birth versus cesarean section. Cesarean babies have more peanut allergies, theoretically, because they did not get the bacterial exposure in their microbiome at birth [inaudible] colonized differently. I think there may be all sorts of different endpoints and ramifications related to the microbiome, not just [inaudible] chemicals or metabolism, chemicals that pre-systemic or intro [inaudible] circulation type of issues, but there may be other more clinical endpoints as well.

Audience Question: [Inaudible] Okay, I’m good. Okay, it is really just about the food packaging and we talked today about chemicals that are handled the way that most of us have thought about, they’re metabolized, or they’re bound to things, and they get absorbed. My question really has to do, and this is relevant to the microbiome, is any, what work is being done about looking for transference of nano silver from food packaging to either the developing fetus or across the placenta or the impact on the microbiome itself? Information?

Faustman: Yes. Who do you want to answer that? I can address a bit of that.

Audience Member: It is a generic question, Elaine. I’m just very curious about this.

Faustman: Yes, I think a lot of people are. So, the best studies that I have been looking at, not specifically from food processing but actually from silver nanoparticle exposure and then looking at the genetic diversity of the gut microbiome, are studies from the University of Michigan. And
they actually, much to their surprise, they were not seeing very dramatic shifts in the portfolio. There are other people that are looking at inhaled and respiratory pathways for silver nanoparticle ingestion and that's suggesting that maybe the respiratory biome is more effected, and one could understand that. So, there is lots of interest in that area. I do not know if the University of Michigan studies are published yet, but that is been funded as a part of the nano consortium studies out of NIEHS.

**Neal-Kluever:** I am not able to talk about specific products in this forum. That would lead into the policy and regulatory domain that I need to veer away from. I can say there is a nanoparticles working group that's FDA-wide. They are looking into nanoparticle-related issues. So, I would be vigilant on what the Agency puts out regarding nanoparticles, and that about is the extent to which I can say on that topic.

**Goering:** We have been talking about some challenges in this kind of risk assessment and some implications of things like factors like the microbiome and social and cultural lifestyle issues, chemical exposures. Here is another area to consider, and that is how can we, or should we, integrate disease models into these kinds of exposures, because typically, many of our risk assessment are done with healthy physiology criteria used. Susan, maybe, you talked about the dermal exposures, so there are intact skin issues but then there are may be chronically or subchronically upgraded skin issues in infants and maybe, yes, we will just keep it at infants for now, and I think another issue for the life stages that we have been talking about, infants to adolescence, would be asthmatics. How would we factor asthmatic physiology into the risk assessments of the sensitive life stages? Susan, since you were talking about consumer products?

**Felter:** Sure. That is really a good question because when we think about diapering, you cannot think about diapering without diaper rash. Every child will experience it at some point, some more than others. So, there is clearly a potential with severe rash and it could also be other skin conditions in adults for which the same is true, that is the potential for a chemical to be absorbed through the skin is higher. Many risk assessments done by P and G and other consumer companies start with an assumption of 100% dermal penetration, and very often, one of the points that I really want to emphasize is, if you start with assuming 100% dermal penetration and you don't have it adequate margin of safety, there's nothing wrong with going back and refining that, right? With the majority of healthy skin, most compounds are not that well absorbed, [inaudible] is actually well absorbed. So, it doesn't make that much difference whether it is in intact skin or you have diaper rash or other disease condition. We assume 100% for all skin.

Other compounds, that is not true. That can be quite a big difference depending on the [inaudible] properties of the chemical that you are looking at to define the quantitative impact of diaper rash. So, we have developed some models, as have other companies, that have various ways of disrupting the [inaudible], and you could do it, for example, by tape stripping, removing the whole upper layer of the [inaudible], which would dramatically change barrier function of the skin. And even under those conditions, one of the papers published by a colleague of mine last year or the year before was looking at a [inaudible] phosphate that was very poorly absorbed through intact skin, I think it was less than 1%. And the question is, what happens if you have severely compromised skin? Is it going to be 100%? That could result in a huge difference in the exposure. Even with skin were the barrier property was significantly altered it was still less than 5%, significant difference from healthy skin that needs to be taken into account for risk assessment but certainly not 100%. So, I think it goes back to case-by-case. If you are looking at something, if you start with assuming 100%, then there is no more work needed, and that is
where questions of pharmaceuticals or things intentionally applied to compromised skin really do need to consider that on a chemical-specific basis.

**Goering:** Any other? Gary.

**Ginsberg:** On that subject, this is more on the environmental side of things, but we often get asked, how if you can bathe or shower in the water, if you cannot drink the water? Certainly, something that is volatile that gets into the shower breathing zone airspace, the answer is pretty simple. But [inaudible] metals that are not very volatile. What is, Manganese, for example, [inaudible] or arsenic in drinking water, what is the dermal risk from sticking a baby in a bathtub? Permeability is not that different from adults, so I would like to see the [inaudible] stratum corneum just to help inform the bathing and showering scenario, which we don’t really have a lot of empirical data for most chemicals on.

I would also, in respect to Peter’s question, I sat on National Academy panel called, that ended up [inaudible] and decisions [inaudible] going forward, and we talked a lot about interaction with background of these processes, so when you are thinking about the dose response for a chemical, it is not just necessarily the chemical by itself, not necessarily the chemical with other chemicals that you interact with, but also can the chemical interact in pathways, looking upstream perhaps, that the disease is also hitting on? So, for example, cadmium and risk to the kidney and GFR impacting, we know that chronic kidney disease in just people getting older, you'd [inaudible] GFR [inaudible] filtration, and cadmium also to [inaudible]. Specifically, cadmium impairs GFR and the interaction, how much more quickly does cadmium age renal function is a very relevant question in the population that is aging and has challenges and has a fair number of people who need dialysis. [Inaudible] interacting to better understand the risk.

**Goering:** Elaine, any comments on disease models, integrating them?

**Faustman:** That is interesting that you asked me because in the last example, several years ago Bob [inaudible] and I forget who else were co-authors on that paper that looked at age-related impact on uncertainty factors. Particularly they emphasize the age aspect of this and the idea of a high proportion of the population that had kidney failure was one of the examples they use. We were interested in those because for some natural toxins, like [inaudible] acid, the basis for the safety factor for, this is a toxin from harmful algal blooms that gets into food products, well, food, seafood, and the issue is there is the whole safety assessment is driven by adequate kidney function. I think we forget how many of these compounds, I think, Gary, you mentioned this, I think these disease models for that are very important. We automatically already think about the immune [inaudible] because that is a huge issue for a large number of people in our population and of course I cannot remember, did somebody say asthma? Anyway, asthma is frequent in our population, so when we set safe models for air pollutants, we consider asthmatics in the identification of sensitive population. So, yes. But we need to do more.

This also relates a little bit to an earlier question which was one of the cases where I had my hand up, but I don't think you could hear me, speaking madly here in Seattle, and that was this issue of how do we account for impacts that are delayed? And the questioner asked about how good are we at assessing those? Obviously we are starting to touch on function and unless we don't look, unless we look for function or challenge function, then we often times are not able to pick up these differences, and that is the place with kidney impacts that if you have challenge test later on or immune challenge test, there's a lot of that being done in the nanotoxicity world by [inaudible] and others. It’s challenged our exposed animals that don't look different, are they still able to mount the kinds of immune responses that would be normal in normal infections and
things? So, I think that is a huge area for us to look at and making sure we get adequate representation there. So, thanks, Peter.

**Goering:** Thank you, Elaine. I think we are about ready to wrap up. We have time for more one more question. It is from online. I do see another online comment here, it says, excellent meeting. That is a good sign. Here is a question that has been up here since the beginning and I wanted to get it in. It can be our last question that the panel responds to. It has to do with endpoints, it has to do with relevant endpoints that we monitor in children, and the example is concerning clinical trials in children with pharmaceuticals and the issue of reversible developmental delays due to body weight reductions that are commonly found in juvenile tox studies. What are the relevant endpoints to monitor? Is that clear?

**Ginsberg:** I am not sure exactly what the body weight changes this question is talking about, but there is an emerging literature on small for gestational age, low birth weight, this is on the fetal side. Then, smoking, risks with smoking, and rebound obesity. So, part of the concern about increased obesity statistics is, are from things like [inaudible] compounds that can cause developmental delays in utero and you see the growth curve postnatally, the catch up and the overgrowth leading to obese mice six months down the road. So, there can be, we are thinking that metabolic setpoints for all sorts of energy utilization pathways can be set up in utero and that if that developmental program is affected by constriction of blood flow to the fetus or some other growth retardation, that the postnatal program is going to be changed. I don't know if that is what where this question is going, but that is just an observation that it is sort of endpoints what we need to really be aware of.

**Goering:** Susan?

**Felter:** I can't speak specifically to the exact question on pharmaceuticals, but if you were to see decreased pup weight in a tox study and it was associated with maternal toxicity and that was a driver behind it, then that would be different, but if you had a conserved or decreased pup weight that would serve as the basis for the risk assessment and you would use that as the starting point to ensure that you are being protected for that. Now, for pharmaceutical development, where you're trying to balance efficacy and safety, I don't know if there are situations where that might be viewed differently such that that becomes a more critical consideration in how one would balance that versus the desired efficacious outcome.

**Goering:** I think we will conclude there, and if people could just stay seated for one more minute. I wanted to thank our speakers for their fantastic presentations today on a very interesting topic, a topic that I was not that familiar with, but I think I can speak for a lot of people that we learned a lot today from the presentations and from the discussions, and I would like to thank Elaine and April and Allen, I think you were the organizers of the, you were the, a larger group that we shared at the beginning, so I want to thank that organizing group and the chairpersons today for their efforts. And thank Betty Eidemiller and her staff from the Society of Toxicology for doing much, much work that happened before the actual meeting that made it a [inaudible]. And the FDA folks in the upstairs that helped us and ran up and down the stairs to save the day a number of times.

I just have one more slide for you. Could you advance? There you go. We are planning to have another series of colloquia in the next year, late 2016 and 2017. If you would like to email us a topic for consideration, I think the committee would welcome that, and I just wanted to remind everyone here and online that the colloquia materials for all of the colloquia are online at the SOT website, the recordings and the slides and the captioning text. And best of all they are
available at no charge. Thanks to everyone. Thanks to our speakers again, and Elaine, thank you for anticipating from a distance.

Faustman: Thank you.