UNITED STATES OF AMERICA

DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

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CENTER FOR TOBACCO PRODUCTS

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RISK ASSESSMENT OF TOBACCO PRODUCTS: A PUBLIC WORKSHOP

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November 15, 2016
8:30 a.m.

The Marriott Inn and Conference Center
University of Maryland University College (UMUC)
3501 University Boulevard East
Hyattsville, MD 20783

FDA:

CAROLYN DRESLER, M.D., M.P.A.
Associate Director for Medical and Health Sciences
Office of Science
Center for Tobacco Products

DAVID L. ASHLEY, Ph.D.
RADM (retired), U.S. Public Health Service
Director, Office of Science
Center for Tobacco Products

SUSAN CHEMERYNSKI, Sc.D., M.P.H.
Lead Toxicologist, Division of Nonclinical Science
Office of Science
Center for Tobacco Products

Professional Video Associates, Inc.
2515 Saint George Way
Brookeville, MD 20833
301-924-1556

SESSION 1: HAZARD CHARACTERIZATION

LYNNE HABER, Ph.D., DABT
Toxicology Excellence for Risk Assessment Center
University of Cincinnati

PAIGE N. WIECINSKI, Ph.D., DABT
Altria Client Service

WILLIAM H. FARLAND, Ph.D., ATS
College of Veterinary Medicine and Biomedical Sciences
Colorado State University

GLENN RICE, Sc.D.
National Center for Environmental Assessment
U.S. Environmental Protection Agency

KATE GUYTON, Ph.D., DABT
International Agency for Research on Cancer

JULIA HOENG, Ph.D.
Philip Morris International

SESSION 2: EXPOSURE

PAOLO BOFFETTA, M.D., M.P.H.
Icahn School of Medicine at Mount Sinai

NEIL E. KLEPEIS, Ph.D.
Graduate School of Public Health
San Diego State University
Center for Behavioral Epidemiology and Community Health
(C-BEACH)

RYAN J. POTTS, Ph.D., DABT
RAI Services Company

BAHMAN ASGHARIAN, Ph.D.
Applied Research Associates, Inc.

GEORGE M. WOODALL, Ph.D.
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency

SESSION 3: POPULATION SUSCEPTIBILITY

DALE HATTIS, Ph.D.
Clark University

WEIHSUEH A. CHIU, Ph.D.
Texas A&M University

PAOLO BOFFETTA, M.D., M.P.H.
Mount Sinai School of Medicine

M E E T I N G

(8:30 a.m.)

DR. DRESLER: The nifty clock says that it's 8:30, so we will start on time. Welcome, everyone, to this workshop. I'm very glad to have you here.

We will start out with myself. I'm Carolyn Dresler. I am in the Office of Science at the Center for Tobacco Products, and I will be moderating today's session, and so you'll be hearing from me throughout today and tomorrow.

But I would like to start, first of all, with opening remarks from our Office Director, Dr. David Ashley.

David.

DR. ASHLEY: Wow, Carolyn, that was a short introduction.

(Laughter.)

DR. ASHLEY: I'm David Ashley. I'm Director of the Office of Science at CTP. And first off, I'd like to welcome everybody and thank you for being here, both the folks that are in the room and the people that are online doing the webcast. There is a webcast today, and so there are a lot more folks watching this than are actually in the room, and that's always exciting.

FDA is committed to using the authority that's been

granted to us by Congress to promote public health by reducing the disease and death that results from tobacco use. Since 2009 we have had the authority to regulate the manufacture, marketing, and distribution of cigarettes, cigarette tobacco, roll-your-own, and smokeless tobacco, to reduce harm across the population. And then in August of this year, a few months ago, the deeming rule went into effect, and deeming has extended FDA's authority to tobacco products which were not previously regulated.

We're committed to a consistent, transparent, and predictable process for the review of tobacco product applications. To do this, we have tried to learn as much as possible about the products we regulate and the science that supports those decisions.

Over the past 7 years, we've held numerous listening sessions with interested parties. We've met directly with manufacturers to answer their questions about the review of specific products. We've participated in visits to farms, manufacturing sites, and laboratories. We've held meetings with TPSAC, our Tobacco Products Scientific Advisory Committee, and we have held scientific workshops.

This workshop is designed to open the discussion regarding

available data and approaches to inform the risk assessment of tobacco products and to support tobacco regulatory science.

The three main objectives of the workshop, and they are to identify the available data to characterize tobacco product users; identify the available data and risk assessment methods to characterize exposures and health risks associated with different types of tobacco products, products within the same class as well as tobacco product constituents; to identify areas of research and method development which may further strengthen the knowledge and approaches regarding tobacco product risk assessment.

Now, the purpose of this workshop is not to debate whether one particular product or product class presents a lower hazard than another. That's not why we're here today. It's intended to discuss the scientific approaches to addressing those questions and to increase our understanding of how best to make those comparisons. My hope is that we will all go away from this workshop with a clearer understanding of the methods that can be employed, the data that can inform those evaluations, and the factors that should be considered when we make those evaluations.

There are many ways that applicants may be able to address

the statutory requirements for FDA to make the findings that are required in product review. We do not expect and are not seeking consensus here. We're seeking that by sharing our knowledge and different scientific perspectives, the information submitted to FDA will be improved, and FDA can make the soundest regulatory decisions possible.

So that's kind of the format, and that's the way I see what we're doing today, and so I welcome all of you. I'm glad you are here. I'm hoping this will be a really productive and educational couple of days. I believe it will be very educational for us. And thank you again for all of you being here.

DR. DRESLER: If I were to give a good introduction to Dr. Ashley, it would take like probably an hour.

(Laughter.)

DR. DRESLER: But I will say for many of you that are speaking at this, and maybe some of you who are online, so I will say my personal opinion of David is that he knows more about the tobacco products than anybody outside of industry, and that's how I usually describe him to my friends. And he's shaking his head, but that is what I think of him. So besides all of his scientific accomplishments, he's a phenomenal fount

of knowledge and a terrific mentor. So David, I'm sorry. I could go through all the other awards you have, too. Anyway, he's the leader, our fearless leader of our office.

And then we have another aspiring fearless leader, and the point person for this workshop who has done the work to pull together the team within the Office of Science and to pull all of you together as speakers and organize this amazing conference is Dr. Susan Chemerynski, who is a lead toxicologist within our Office of Science, and she will give some more overview of toxicological risk assessments.

Susan.

DR. CHEMERYNSKI: Thank you so much, Dr. Dresler, for the introduction. And thank you also, Dr. Ashley.

Good morning, everyone. As Dr. Dresler said, I am Dr. Susan Chemerynski. I am a lead toxicologist in the Division of Nonclinical Science in the Office of Science at CTP and also the science lead for this workshop.

I would really like to start first by thanking the group of scientists and staff at CTP that worked with me to develop and plan this workshop, many of whom are here today, and all of our presenters, panelists, and guests both here in the room and with us online, for your participation. We're really grateful.

I'm going to spend a few minutes this morning giving you an overview of the purpose and objectives of the workshop as well as where we will be heading throughout the scientific session, so for the next few days.

First, let me briefly acknowledge my disclaimer for this presentation as noted here on this slide.

So let's focus for a few minutes on the scope of this workshop. As Dr. Ashley articulated, the workshop is designed to open the discussion regarding available data and approaches to inform the risk assessment of tobacco products and to support tobacco regulatory science, and therefore necessitates that we focus first on the science of risk assessment that has been applied and has evolved over the past decades. As we consider here the framework for risk-based decision making that has been published by the National Research Council, we will be focusing on Phase II, Planning and Conduct of Risk Assessment, and particularly on the risk assessment stage highlighted here for the focus of this workshop.

As many of you know, the purpose of a human health risk assessment is ultimately to characterize the nature and magnitude of health risks to humans from the agents of concern. Traditionally, this is accomplished by completing each step of

the risk assessment paradigm:

- Hazard identification, which identifies the adverse health effects associated with the agents of concern;

- Exposure assessment, which characterizes the exposures and doses incurred by the population of interest;

- Dose-response assessment, which characterizes the relationship between the dose and the probability of adverse effects in the range of doses identified in the exposure assessment; and

- Risk characterization, which characterizes the nature and magnitude of risk associated with the exposure conditions as well as the degree of uncertainty present.

Applying risk assessment approaches specifically to the consideration of tobacco products can inform tobacco regulatory science efforts, and we will have presentations and a discussion over the course of the workshop regarding both established and emerging approaches for risk assessment that may inform the risk assessment of tobacco products.

As further background for our discussion, the FDA now regulates tobacco products, including but not limited to cigarettes, all cigars, dissovables, electronic cigarettes, hookah, roll-your-own tobacco, pipe tobacco, smokeless tobacco,

and future tobacco products that meet the statutory definition of a tobacco product. Please note, we do not regulate accessories of newly deemed products.

In thinking about the hazards and health risks associated with tobacco product use, there are numerous characteristics that are present in and produced by tobacco product use that may contribute to human health risk. This includes identified harmful and potentially harmful constituents, or HPHCs, ingredients such as flavors, product material components, and design features.

In thinking about data and risk assessment methods that can be employed in the evaluation of tobacco product exposure-related health outcomes and risk, we can consider the conceptual model for exposure-related disease across the source of the health outcome continuum, as it relates specifically to tobacco product-related hazards, exposures, and health outcomes.

Depending on the type of tobacco product being consumed, a user may be exposed to numerous constituents via the inhalation, dermal, or oral pathways and, depending on constituent- or mixture-related toxicokinetics and toxicodynamics, may progress to individual or multiple acute or

chronic health outcomes.

Given the available data and approaches that are employed, there will be multiple sources of uncertainty as well as many factors that may contribute to variability along the continuum, including concentration factors such as the amount of product and the product characteristics, exposure factors such as product use and exposure pathway, and dose-response factors such as age, genetics, and metabolism. The identification of the hazards combined with the exposure conditions across the continuum will equate to the health outcome risk.

With that background in mind, we developed the workshop with three primary objectives, as Dr. Ashley mentioned:

First, to identify available data to characterize tobacco product users.

Second, to identify available data and risk assessment methods to characterize exposures and health risks associated with different types of tobacco products as well as tobacco product constituents, keeping in mind that we're talking about different types or classes of tobacco products and also about products in the same class that have different characteristics.

And third, to identify areas of research and method development which may further strengthen knowledge and

approaches regarding tobacco product risk assessment.

Over the course of the next few days, we will have presentations and panel discussions from members of academia, governmental agencies, industry, and other organizations. Today we will begin with a session on hazard characterization followed by a session on exposure and a session on population susceptibility. Tomorrow we will be focusing all day on risk characterization, first with an overview session and then followed by sessions on cancer and non-cancer approaches, mixtures, and uncertainty and variability.

The sessions have been architected with the risk paradigm in mind and include presentations that address key questions regarding available data and risk assessment methods that may be applicable to the characterization of hazardous constituents and complex mixtures, tobacco user populations, tobacco product use and exposure, susceptibility factors to evaluate the cancer and non-cancer risks from exposures to individual constituents and to complex mixtures from different types of tobacco products, and approaches to quantify and incorporate sources of variability and uncertainty.

With that, I look forward to the presentations and discussions over the next 2 days. I would like to thank you

all again for your participation and would like to welcome back Dr. Carolyn Dresler, who will introduce our first session and our first session speaker. Thank you for your attention.

(Applause.)

DR. DRESLER: Okay, so before we jump into this exciting set of lectures that are coming up, let me do those more mundane things, like where's the bathroom?

(Laughter.)

DR. DRESLER: So, you know, as you came down this hall, if you go for the bathroom, if you just go back down the hall and turn right, don't go up the stairs, turn right, and the men's and women's are right there.

The food across the hall, I wish that was ours, but it is not. Please don't help yourself to it; they get upset. So I'm sorry, but we don't have food and drink. We do have water that's around the table on the outside, if you would like that.

And also this session is being recorded, and we have a large number of people who are watching it online, so that's why you'll be seeing the cameras and the pointers and everything, because this is being recorded, and many people are watching it online.

So with that, let's go ahead and start into our first

session, which is Hazard Characterization, and we'll start it out with Dr. Lynne Haber from the University of Cincinnati Toxicology Excellence for Risk Assessment Center, and she will be speaking on Hazard Characterization: Overview of Issues and Tools.

Lynne.

DR. HABER: Well, thank you. I'd like to thank the organizers for the invitation to speak here and the opportunity to share a bit about issues and tools related to hazard characterization. I'll be talking about some of the recent developments in hazard characterization, spending a bit of time talking about some of the issues and tools that can help us address those issues, and then just briefly talk about some data sources and resources.

So it was useful. I was glad the previous speaker shared this slide with you also. She talked a lot about the central part of the risk assessment paradigm. I just wanted to note that one important addition in the National Research Council Science and Decisions Report was this Phase I on problem formulation and scoping.

So when we do risk assessments, we are doing them in order to solve an issue, to address a specific problem, and the

nature of that problem defines how we do the risk assessment, and that was part of the workshop objectives. And then an important part after we do the risk assessment is making sure that the risk assessment did indeed inform those issues and helped to discriminate among risk management options.

So now moving into some of the methods and developments in the field of hazard characterization. One important aspect is the definition of what is adverse, and this may seem like a very simple question, but we're not looking to protect against every single change. The body has homeostasis; we're constantly changing as we go throughout our day, but the goal is to protect against adverse health effects, including their precursors.

And there are a variety of different definitions across organizations, but they have in common basically the goal to -- the definition of adverse includes changes that affect the organism's performance as well as reducing the ability to respond to additional challenges.

I also wanted to point out this recent publication from the Society of Toxicological Pathology, where they not only included a definition of adversity but provided a lot of additional guidance on how to distinguish what is adverse.

An important area of hazard characterization is consideration of mode of action. We distinguish mode of action from mechanism of action, where mechanism of action is a very detailed understanding at the biochemical and molecular level -- so that includes all of these very complex events in this diagram -- while mode of action focuses on a few key events that are obligatory steps and are dose and rate limiting.

Mode of action is important both in the hazard characterization and determining the conditions under which the chemical causes the effect or the mixture causes the effect as well as the nature of the dose response.

And particularly related to smoking, the consideration of the impact of the route of exposure is important. So you may have a chemical that is likely to be carcinogenic to humans by inhalation, such as certain metals where they deposit in the lung, but they're not likely to be carcinogenic to humans by oral exposure because they're not taken up from the gastrointestinal tract.

Similarly, mode of action is an important part of the dose-response assessment, so in this example identifying this chemical as likely to be carcinogenic to humans at doses that cause cytotoxicity and regenerative cell proliferation and not

likely to be carcinogenic to humans in the absence of this key event of cytotoxicity. And so this incorporates mode of action information to say that only under certain dose conditions is the chemical causing cancer.

An important part of evaluation of mode of action is using the Bradford-Hill criteria, which have had a number of different iterations and evolutions. The most recent that I'm aware of are these evolved Bradford-Hill criteria that were published a couple of years ago.

And one important thing about this publication is that it gives some priority of importance for the criteria. It emphasizes looking at biological concordance. So does the hypothesized mode of action of mode make sense based on what we know about biology?

Looking at the essentiality of key events, so if you knock out a specific key event, does that knock out the downstream events?

And then as a less important consideration, the empirical evidence, so looking at concordance of the dose response, the temporal relationships, and the incidence both among key events and between the key events and the adverse outcome. So do the key events happen in the expected order based on dose response,

time considerations, and incidence? And are they occurring before the adverse outcome?

And then consistency: So is the pattern of observations across different test systems consistent with what would be expected based on the hypothesized mode of action? So note that we're not looking for complete uniformity, but is it consistent based on the mode of action?

An important part of doing a mode-of-action evaluation is comparing the weight of evidence across alternative hypothesized modes of action, and using tables like this is very important for transparently laying out the data. So you have the evolved Bradford-Hill considerations and then information on the supporting data, inconsistent data, and missing data. And it's also very important to lay out what that missing data are because that helps to identify what key studies would be help to address uncertainties.

Mode of action is also used to help evaluate human relevancy of different effects. This framework that I'm showing you was developed by the International Life Sciences Institute and the International Programme on Chemical Safety, and it consists of three questions. The first thing is evaluating the mode of action according to the criteria that I

showed you, and is the weight of evidence sufficient to understand the mode of action in animals? If yes, then we ask can human relevancy of the mode of action be reasonably excluded on the basis of the fundamental qualitative differences in key events between animals and humans, so based on the basic biology? And can the human relevancy of the mode of action be reasonably excluded on the basis of the quantitative differences in either kinetic or dynamic factors between animals and humans?

And a recent development in an area related to mode of action is the consideration of adverse outcome pathways, or AOPs. So the mode of action includes the chemical-specific information on toxicokinetics. While the AOP is considered chemically agnostic, it's independent of the chemical, and it's specific to the biology. So that's the toxicodynamic aspect, and it begins with a molecular-initiating event and then follows a series of key events.

The advantage of looking at AOPs is that they're modular, and once you've established it in one situation and you identify the molecular-initiating event for a new situation, you can apply the AOP. It is important to note that just because a molecular-initiating event is occurring, it does not

mean that all the downstream events are occurring.

An important aspect: The AOP is also looking at the response-response relationship. So what degree of change in one event results in what degree of change in the downstream events? And that determines whether you're going to be actually getting your adverse outcome.

Another important development is systematic review. The goal of these is to improve the rigor, reliability, and replicability of assessments, and the way this is done is using transparent procedures to identify, evaluate, and synthesize the results of relevant studies and use predefined and structured criteria. So, again, the goal here is to improve transparency.

There are a number of guidance documents that have been developed. A lot of the work so far has focused on systematic literature searching methods, but there are a number of case studies under development and additional work being done to develop methods for the systematic evaluation of the data, study quality, and integration of data across different data streams.

Given the importance of cancer in the evaluation of tobacco products, I wanted to note a few trends in genotoxicity

analysis, including integration of genotoxicity endpoints into in vivo studies so that you can directly compare the effects seen with the genotoxicity data and the effects in the in vivo study; use of transgenics both for screening and for mode of action information; use of flow cytometry where you can evaluate thousands of cells to increase sensitivity; quantitative analyses using computer programs; and then evolving guidances for genotoxicity testing.

I'd like to now move on to consideration of issues and tools that can be used to solve those, address those issues.

So in thinking about what sorts of issues there are and looking at the literature, I identified some of the challenges that are part of the evaluation of tobacco products and here, in particular, related to smoke. One of the challenges is that it's a very complex mixture. Not only is it a mixture of many different chemicals, but it includes both gases and particulates, organics absorbed onto the particulates, that the actual nature of the smoke changes with time, as I'll be talking about in moment.

Part of what we look at for extrapolating from animals to humans is the deposition in the lung, but there are a number of mechanisms that affect that deposition, and then the behavioral

characteristics that also result in variability in the nature of the smoke and the type of exposure.

So in extrapolating from animal data to humans, it's important to consider differences in the respiratory tract structure and the implications of those differences, particularly between rodents and humans, for what's actually deposited in the respiratory tract and the region of the respiratory tract to which that's deposited.

This is something that we do routinely in the context of risk assessment for environmental chemicals, and the way we do this is by calculating what we call the human-equivalent concentration, and that human-equivalent concentration, or HEC, is defined as the concentration that people would be exposed to under the exposure scenario of interest that would result in the same dose to the target tissue of interest of the active form of a chemical that the animals received under their test conditions. So in order to do this, we look at the exposure regimen, the type of agent. And for particles, we need to describe the particle size distribution; for vapors and gases, the water solubility and reactivity; and then if there are systemic effects, looking at the blood-to-air partition coefficient; and then we typically do this fairly crudely, so

looking at the region of the respiratory tract versus a systemic effect.

And EPA has published methods and equations for doing these extrapolations from the animals to humans for gas exposure for the tracheobronchial region.

This is an example showing that the relative deposition and the relative dose is based on how much of the chemical is inhaled, so the minute volume, and then that's normalized based on the surface area of the region of interest. And then for extra-respiratory effects, the systemic effects, the normalization is based on the blood-to-air partition coefficients because that's what determines the concentration in the blood at steady state.

And there are much more sophisticated, much more complex methods that can be used to address this issue, such as computational fluid dynamic modeling. And this is an example of the work that was done by Julia Kimbell and colleagues for formaldehyde, where you can look at a very detailed level at the data.

The state of the science for particle dosimetry is using the program called Multiple-Path Particle Dosimetry model, or MPPD. I was happy to see that Bahman Asgharian is on the

schedule later in the meeting, and he and his colleagues have developed this wonderful free software which is very powerful but can also be used for simplified approaches. It addresses a number of species and can also look at different strains within the rodent species.

And this is just an example of the types of output you can get, looking at the regional dosimetry.

One of the important issues for addressing, in particular, smoking-related exposures relates to the duration of exposure. So you can imagine a specific exposure incident which may last 15 to 20 minutes, but that's repeated several times over the course of the day, and then that's maybe repeated for many years.

So in comparing different products, you're maybe saying, well, what's the appropriate limit I might be using? Should it be an acute or a chronic limit? If I'm looking at animal studies, how do I extrapolate from the duration of the animal study to the actual exposure scenario? Often this extrapolation is based on the time-weighted average exposure which is related to the cumulative dose, but that's not necessarily an appropriate dose metric. And so this just shows, for carbon monoxide, that for the same cumulative exposure of 2,000

ppm-hrs, having different exposure scenarios can result in dramatically different effects.

And we've recently published a framework that helps to address this issue of intermittency. So I know this is a busy slide, and you can't see the details, but this is just to show that the framework can use information on the kinetics and the dynamics of the chemical to help you address when you have an intermittent exposure, in addition to comparing the exposure with an exposure limit that's the most relevant to that specific single exposure; whether you also need to be comparing relative to the chronic exposure limit; and also addressing the issue of whether area under the curve, the total cumulative exposure or peak exposure is the more appropriate dose metric.

Categorical regression is another tool that can be used to help do these extrapolations across different time periods. It's available as CatReg from the EPA as part of its Benchmark Dose Software package. And categorical regression is a method for considering concentration, duration, and severity of response relationships, and it can be used to extrapolate across different exposure durations as well as considering how the concentration response of different severities are related. And Rick Hertzberg is here in the audience and has done a lot of the

research on that.

Oh, gosh, I need help getting this back to being advancing.

UNIDENTIFIED SPEAKER: Hit escape.

DR. HABER: Hit escape. There is no escape.

DR. DRESLER: Yeah, you'll see it.

DR. HABER: Thank you.

(Laughter.)

DR. HABER: Okay. Thank you to the magicians.

So PBPK modeling, physiologically based pharmacokinetic modeling, is also another powerful tool that can be used to improve assessments. It can be used to help improve extrapolation across species. It can be used to address issues of duration of response as well as doing route-to-route extrapolations.

And this slide just shows the importance of choosing the appropriate dose metric. The green are drinking water studies, blue are inhalation studies. And if you've chosen the appropriate dose metric, that means that dose predicts response, and you can see here, for area under the curve, you have the same doses and dramatically different responses. Well, for the peak concentration, you have a nice linear regression, so here it supports the choice of peak

concentration as the appropriate dose metric.

I know there are additional talks later this morning on both mixtures and toxicity testing in the 21st century. I just wanted to give one example of how these two can interact. One of the challenges is that, for mixtures, you can make -- it's just impossible to test, in vivo, all the different combinations of mixtures and dose combinations, combinations of different chemicals, but in vitro testing can help us rapidly screen many different combinations of mixtures. And the example I'm showing here is from testing that was done in the aftermath of the Deepwater Horizon explosion, where they were screening dispersions to identify which were of lower toxicity.

So moving very briefly into different data sources and resources. One of the challenges for evaluating tobacco products is that many of the components have little data, and there are a number of tools that are being developed to help address such issues, including read-across, where a key issue is identifying the appropriate analog or categories of analogs, and there are a number of tools for doing that. In identifying those analogs, it's important to address these issues and make sure that you've got an appropriate justification for your choice of analogs or your category definition and that you've

evaluated the overall weight of the evidence.

Another approach that's often used for addressing data-poor situations is route-to-route extrapolation. If you're doing that sort of extrapolation, it's very important to consider whether the chemical is causing or the product is causing effects at the portal of entry and what the nature of those effects are, because if there are portal-of-entry effects, then the route-to-route extrapolation is not appropriate.

And then some other databases that can be of utility. The ITER database is a compilation of chronic toxicity values, both ones that have been developed by world health groups as well as independently developed values and values that have been published. It includes information on the key decision points for these risk values, and there's now a standing committee that conducts quality assurance reviews of published values, including DNELs that have been reviewed by ECHA.

Risk IE, or the Risk Information Exchange, is a way of communicating in-progress risk and toxicity assessments and specific issues. So if you're working on an individual assessment or addressing an issue, you can go onto this database and see whether there are others who are working on

that issue and communicate with them. You can also post your own issues and use that as a way of finding other people you can talk with.

And then, finally, a dose-response framework that was developed as a tool. It was part of a workshop series that occurred in response to the Science and Decisions Report from the National Research Council, and this framework organizes a variety of risk methods, including case studies illustrating those risk methods by different problem formulations, and then also includes guidance documents that are related to those risk methods.

So with that, I'll stop and thank you for your attention. And I look forward to the panel discussion.

(Applause.)

DR. DRESLER: Thank you. Okay, a great start.

So now our next speaker is Dr. Paige Wiecinski from Altria Client Services, and she will be speaking on Weight-of-Evidence Approach for Risk Assessment for Tobacco Products.

Dr. Wiecinski.

DR. WIECINSKI: Well, good morning, everyone. First, I would like to thank CTP for providing the opportunity to come and speak to you all today. As introduced, my name is Paige

Wiecinski. I am a toxicologist with Altria Client Services, and I'll be talking to you today about Weight-of-Evidence Approach for Risk Assessment of Tobacco Products.

Just to ground everyone, for the next approximately 25 minutes, I will define weight of evidence. Many organizations use the term "weight of evidence," but very few actually provide a definition. I'll discuss considerations for tobacco product regulations, what data would be sufficient and how should that data be weighted. I'll provide the history and evolution of the weight-of-evidence approach. I will demonstrate how to apply weight of evidence. We'll go through the four phases as they are described in the literature and use some examples of those phases. And finally, I'll wrap up with just a few take-home messages.

"Weight of evidence" is a frequently used term within the context of risk assessment literature. You'll see it all over the place. If you were to ask any toxicologist how they evaluate a chemical or a product, they'll likely say by using a weight-of-evidence approach. But despite this frequency of use, weight of evidence is not necessarily a well-defined term or formalized concept. Often weight of evidence gets defined within the definition of hazard characterization rather than

separately on its own. One of the few organizations to provide a definition for weight of evidence is the European Chemicals Agency, and that is the definition you see here on this slide.

In general, weight of evidence applies to the process of evaluating the strengths or weaknesses of available data to support a conclusion to a question about risk. Weight of evidence is often applied to hazard characterization, but there are also other places within risk assessment where it can be used.

Now that I have briefly defined weight of evidence, what are some considerations for its application to tobacco products?

Epidemiological data is considered a gold standard for hazard characterization because of its high biological relevance to humans. However, the available epidemiological evidence varies by tobacco product category. What I mean when I say tobacco product category is conventional cigarettes, cigars, smokeless tobacco, e-vapor, etc. Decades of data exists for conventional cigarettes and smokeless tobacco, but little, if any, is available for novel tobacco products such as heat-not-burn or e-vapor.

In the absence of epidemiological data, the question

becomes what types of in vitro or in vivo data would be sufficient to assess the potential hazards of tobacco products, particularly novel tobacco products. Would it be in vitro at the air-liquid interface? Is it animal disease models? Or would systems biology be useful? And finally, how should various types of data and expert reviews be weighted to formulate a decision for tobacco products? The types of data may differ depending on tobacco product category. In some instances, established guidelines by authoritative bodies may be applicable. For example, smokeless tobacco products are used orally. Therefore, it may be appropriate to consider applying some guidelines that are used in food, such as acceptable daily intakes for ingredients.

Now that we all have a definition for weight of evidence and I've provided some considerations for tobacco products, I'd like to briefly go through some history regarding the evolution of weight of evidence.

So in 2011, the National Research Council committee of the National Academy of Sciences recommended that the U.S. EPA develop transparent and defensible weight-of-evidence assessments for the Integrated Risk Information System, or IRIS, program. The NRC did not specify methods but did

recommend a survey of current weight-of-evidence approaches to provide insights to build a more rigorous and transparent approach.

Based on the roadmap provided by the NRC, the American Chemistry Council's Center for Advancing Risk Assessment Science and Policy sponsored a survey of current weight-of-evidence approaches and held an international workshop in 2012 to review the approaches and provide best practices for weight of evidence. The workshop culminated in a review article published in 2013 by Rhomberg et al. that describes these best practices.

Now that we've gone through the history, I will spend a few minutes discussing the best practices as outlined by Rhomberg et al.

Rhomberg et al. defines four phases of weight of evidence based on the survey of current approaches. As we go through each phase, you will note that they each demonstrate three key factors: transparency, defensibility, and the importance of expert judgment.

Phase 1 is the planning and gathering phase, starting with identifying the question, which should not strive to identify only a correlation but should be causal in nature and

demonstrate a direct link between agent and risk. This would be similar to the approach used for adverse outcome pathways, which strive to link defining events to predict the response. Once a question is defined, all sub-questions and hypotheses should be listed, inclusion/exclusion criteria should be defined, the literature search strategy should be designed, and studies should be selected.

Defining a causal question is not always simple or straightforward. As an example, I provide a depiction of exposures and responses which vary temporally.

In the top panel is a demonstration of two stressors that are occurring at the same time, which makes it challenging to determine what stressor caused the response.

The middle panel demonstrates exposures to the two stressors at different times but with overlapping responses. Therefore, it's difficult to separate and differentiate the responses according to the stressor that caused it.

And the last panel demonstrates the toxicologist's dream --

(Laughter.)

DR. WIECINSKI: -- where all exposures are separated by time, and the link to the response is clear. If all examples

were like this, the toxicologist's job would be much easier because you can easily identify two separate questions in this scenario. However, defining the appropriate question for the top two panels is much, much more challenging.

Once a question is defined, how should data be gathered? Depending on the breadth and topic of the causal question, data gathering can be rather daunting. For example, a broad health effects question for a commonly studied ingredient identified approximately 24,000 studies, while a more focused question on a second ingredient identified a more manageable 50 studies. Of course, using the inclusion/exclusion criteria defined earlier in the process, the number of studies for further evaluation can be significantly less.

Some exclusion criteria that we have used when addressing health effects of ingredients are studies that used non-mammalian animal models, studies that were not in vivo or in vitro toxicology studies, or studies that examined acute effects only.

When applying the exclusion criteria, we were able to get 24,000 studies down to approximately 1,300 studies for further review. All studies that meet the inclusion criteria, including studies with null or negative data, should be

considered for further evaluation.

Once Phase 1 is complete, we can move to Phase 2, which is where you assess the information gathered in Phase 1. In Phase 2, data is extracted from the studies that were collected in Phase 1. Study quality and study results are assessed and categorized, and study relevance is categorized in accordance with the defined question: How would you assess and categorize study quality? One way is to apply Klimisch criteria.

Klimisch scores are well established and still recommended in the literature as a best practice for defining study quality. Klimisch scores are defined such that the lower scores denote higher study quality, with the highest quality studies being those performed under GLP and national testing guidelines such as OECD or methods comparable to guideline methods.

Just because a study was not conducted according to GLP or other guidelines does not mean the data cannot be used for weight of evidence, which happens often with older datasets that may predate GLP or mechanistic studies where OECD guidelines do not currently exist. But the methods must be well defined and documented and scientifically acceptable. These studies often receive a quality score of 2. A quality

score of 3 is often assigned when methods are not clear or there are concerns over the study design or materials used. And a quality score of 4 is given to studies that are secondary literature, such as review articles, or provide insufficient experimental details.

To demonstrate the use of Klimisch criteria, I provide an example from a recent publication that assessed studies related to oral exposures of methyl salicylate. Methyl salicylate is the compound that denotes a wintergreen flavor in products.

This is only a subset of the studies reviewed, but as you can see, three of the studies were given a study quality score of 2 because they either met GLP or OECD, but the methods were well documented and scientifically acceptable. The fourth study in this table was given a study quality score of 3 because it was not conducted according to GLP or OECD and there were questions regarding the test substance used. And the fifth study identified was only an abstract. Therefore, it did not provide sufficient information to receive a higher score than 4.

After all of the data is collected and scored, the data must be integrated and evaluated, which occurs in Phase 3. Rhomberg et al. found that Phase 3 was most averse among the

frameworks surveyed. For anyone who has done weight of evidence, this is probably not surprising as Phase 3 highlights one of the biggest challenges in weight of evidence, which is how do you balance the need for a rigorous, transparent, and consistent approach but still provide enough flexibility for each case study that's examined or to evolve as more information becomes available?

So, in general, what types of data are generally considered in weight-of-evidence approaches? Weight-of-evidence approaches often consider chemistry data, denoted here as constituent analysis. For tobacco products such as conventional cigarettes, this may be smoke chemistry, nonclinical data, the in vitro and in vivo toxicology studies, and human measures data such as epidemiology.

As you move up in this pyramid, there is increasing biological relevance but decreasing sensitivity and confidence in the data received. As you move down in the pyramid, sensitivity increases as does confidence in the data, but it's not always clear how or if that data is relevant to humans.

The pyramid on this slide demonstrates a couple of challenges in weight of evidence, the first being how are each of these types of data weighted for weight of evidence? How do

we balance the data with the most confidence with the data that has the highest biological relevance?

And secondly, it is unlikely that a single expert can evaluate each of these data types, which highlights the need for expert judgment and weight of evidence to be multidisciplinary.

With the breadth of data being considered, how should it all be weighted? We all assign weights to literature and data based on our own criteria and expectations for quality. However, we usually all do this up here in our heads. It's not generally written down explicitly, and that's because it's very, very hard to do so. This is very challenging to determine how to weight all of this data in a consistent manner. In fact, there are very few instances in the literature which discuss weighting approaches, one of which is highlighted here on the slide.

That being said, weight should be defined explicitly and consistently applied. Furthermore, as I said on the previous slide, the weight should be balanced and appropriately assigned between mechanistic data and data with the highest biological relevance.

Phase 4 is where conclusions are drawn using expert

judgment. You cannot have weight of evidence without expert judgment, which is one of the key factors noted by Rhomberg et al. The other key factors are transparency and defensibility. Weight of evidence should be transparent and defensible, such that anyone reading cannot only understand the approach but would be likely to agree that the appropriate data was used to formulate the conclusion.

Since expert judgment is so critical to any weight-of-evidence approach, what are the best practices for applying expert judgment? The key point for expert judgment is that the rationale for the conclusions needs to be clearly articulated and include an explanation of why other plausible hypotheses were eliminated. And I cannot stress enough how important it is to have the appropriate expert for each source of data, epidemiologists for epidemiology, analytical chemists for chemistry, and toxicologists for nonclinical data.

I've provided quite a lot of information and hopefully have some wheels turning in regards to how to apply weight of evidence to tobacco products. So with that, I'll leave you with just a few take-home messages.

The goal of transparent weight-of-evidence approaches is to make it easier for someone to evaluate your conclusions. A

transparent, defensible approach should evaluate the causal question critically. Does the question get to causality, and does it provide a roadmap to gather the appropriate data to formulate a conclusion?

Gather and score the data comprehensively. All studies should be considered, not just those that report findings or support your conclusion.

And weights need to be assigned rigorously, consistently, and explicitly. If done appropriately, this will avoid assigning too much weight to mechanistic data. For example, how much weight should be given to harmful and potentially harmful constituent data for tobacco products? What does it mean if HPHC data shows a slight increase in NNK but a decrease in NNN and BaP?

Explicitly state principal and alternate hypotheses and conclusions. If Phase 1 and Phase 2 are done transparently, it is likely that another reader will agree that the appropriate data has been considered, so that everyone can focus on the expert judgment and conclusions.

And finally, multidisciplinary expert judgment should be used. Again, as a toxicologist, you probably do not want me to review and assess epidemiology or chemistry methods. Those are

best left to the experts.

With that, I would like to thank you all for your attention and welcome any questions during the panel later this morning.

(Applause.)

DR. DRESLER: All right, thank you.

Our next speaker is Dr. Farland from the College of Veterinary Medicine and Biomedical Sciences at Colorado State University, and he will be speaking on Bringing 21st Century Toxicology into Hazard Characterization.

DR. FARLAND: Good morning, everyone. I want to thank my colleagues here at FDA for this great and timely workshop and for inviting me to participate. As many of you know, I spent the bulk of my career at EPA doing toxicology and helping to develop the risk assessment process, and we're looking to continue on those sorts of things. I'm in the process of retiring from Colorado State, and I'm doing some independent consulting work, so I'll just leave you with this disclaimer about some of the recent work that I'm doing, but I have no conflicts of interest with regard to tobacco product research or assessment.

So the challenges that we have as we begin to watch this

process of risk assessment develop is to really think about how traditional in vivo toxicologic testing can work for us in this particular instance. And some of the challenges that we face are that these testing paradigms that we've used in the past don't incorporate some of the advances in technology that we have seen and don't provide us with a lot of the mechanistic data that we'd like to have with regard to understanding chemicals. They also don't efficiently assess safety for all of the existing chemicals that we have interest in and can't keep pace with those that are being developed.

So really, this idea of testing and assessing one chemical one exposure at a time, as we've done over the past, really misses the complexity of human biology, and the idea that the human system is something that needs to be assessed as we look at the exposure to complex mixtures in our daily life, so-called exposome, is something that we're trying to address.

So in 2007, the NAS put out a seminal report entitled "Toxicity Testing in the 21st Century," and the idea of the report was really to provide a vision and to indicate a strategy that would allow us to do a look to the future. And while we talked about this as the not-so-distant future, I think all of us recognized that we were talking about perhaps

decades to be able to really bring this to fruition.

The idea was that virtually all of the routine testing would be done in vitro in human cells or cell lines and that we would be looking at a systems approach to evaluating perturbations in a suite of assays that could be run through robotic-assisted methodologies. Ten years on now, we've been able to assess thousands of chemicals in hundreds of assays, and in fact, we're making progress to be able to bring this to fruition.

And so today I'd like to talk a little bit about how we see this evolving and some of the more recent opportunities for us. So we talk about this as needing a systems perspective, and this particular report from the National Academy talked about it in the context of a system that brought about normal biological function, that there was a recognition that in normal biology there can be departures from that normal biological function if there's homeostatic mechanisms that allow us to get to a normal situation when altered cellular responses occur, and these are through adaptive stress responses that maintain homeostasis and bring us back to normal biological function, or in some cases a new normal, if you will, that recognizes the change that has been made but don't

lead to adverse health outcomes.

If we think about exposures that might lead to some type of risk in anti-biological perturbation, we're looking at things that would again impinge on the system and alter the cellular responses.

The so-called adverse outcome pathways that we talked about -- and thanks, Lynne, for introducing us to that concept -- allow us to really think about this from the standpoint of what sorts of responses are we seeing from these various exposures and perturbations, and under what conditions do we overcome homeostasis that leads to this idea of cell injury and adverse health outcomes? So this is the real basis for our being able to bring these types of data into the discussion of risk from environmental exposures.

More recently, there has been a discussion around exactly how we look at this from the risk assessment standpoint. And while there had been a recognition of the idea of molecular-initiating events and the relationship between a series of key events leading to adverse outcomes, there is now a discussion also around the idea of the aggregate exposure pathways that can lead to dosage to targets, and a recognition that while we often focus on an individual exposure, what we're really

concerned about is the total dose to a target in a biological system. And so these need to be taken into account as we begin to think about this continuum between source and outcome.

So when we think about complex mixtures risk assessment, and we'll think about this in the context of tobacco products, there are a number of approaches, and you'll hear from other speakers today about some of these approaches. And as we just heard, there are issues with both constituents analysis and with complex mixtures. But in terms of constituents analysis in mainstream smoke, there have been over 5,000 constituents identified, and as was said, many of these aren't tested. And so we're dealing with chemicals that perhaps we don't have toxicology literature on.

The focus on testing a hazardous subset of these chemicals has been the sort of tried-and-true approach that has been used, and in the case of tobacco smoke, this has been anywhere from nine to perhaps many more of those chemicals in terms of trying to reach some conclusions about hazard. And many of these assume additivity to derive some sort of a composite risk or hazard index that you'll hear about, and these are typically divided into a cancer hazard or a non-cancer hazard. Again, these represent traditional approaches to dealing with a

complex mixture in the absence of actually testing the mixture itself.

If we look at testing of the complex mixtures, again, we could use traditional toxicology or retrospective epidemiology for dealing with these mixtures, as long as those mixtures are very well characterized, and that's the real issue.

On the other hand, in vitro and cell-based assays allow us to increase our efficiency in terms of looking at specific mixtures. We can use gene expression profiling and transcriptomics through RNA seq methods and other evolving methodologies to begin to look at gene expression and pathway activation and biological function as a consequence of testing of complex mixtures.

So while we are, again, 10 years into this discussion, we can talk a little bit about the approaches that have been used and sort of where we're going.

In terms of constituent analysis, the pros have to do with the fact that we're using existing tox databases and traditional risk assessment methods. And again, those can be screened carefully for the quality of the data and the applicability to the decisions that have to be made. We can identify potentially harmful constituents and focus on those,

and that's a good thing.

On the other hand, it uses existing tox data and traditional risk assessment methods, which again don't get at the questions very often about the impact on the system, that it's being exposed to other sorts of things and in some cases the same chemicals. They may not focus on the endpoint of interest in terms of adverse outcomes, simply because of the way that tox testing has been done and the response of animals compared to those in humans and assumptions that we have to make about those particular approaches.

Analytical capabilities today greatly exceed our traditional tox testing capabilities in terms of understanding dose. So we can measure things at much, much lower levels than we can actually test them in traditional approaches.

And typically, we assume no interaction among constituents in terms of these approaches unless they happen to hit the same target, although we don't necessarily know that they are working through the same mode of action.

And we typically ignore endogenous levels of chemicals, which is something that is important if we're going to think about total dose to targets. And so that's an area that needs to be looked at.

Just to expand on a couple of these issues, we've already heard about the difference between understanding of portal-of-entry effects and systemic effects. This becomes particularly important as we begin to look at some of the tobacco products, and the route of exposure becomes particularly important in terms of understanding the response of various types of tissues to these chemicals that are providing exposure.

There can be direct versus indirect effects, and we know that the same chemical is not necessarily producing effects in a system, that a response in one tissue can actually give rise to a response in another tissue as opposed to the chemical itself.

And finally, with regard to adverse outcomes, we typically dichotomize this idea of cancer versus non-cancer effects. Yet, the underlying toxicology in many cases may be similar, and it's a question of the rationale of generating a particular response with regard to dose or duration or particular impact on the system that would give rise to one or the other of these responses.

The approaches that we use in risk assessment tend to be dichotomous in a sense that we are not yet using a unified approach, although the Academy has suggested that we attempt to

do this.

And typically, we use a linear assumption below a point of departure, whether we're talking about cancer or non-cancer, as we apply uncertainty factors to non-cancer effects. So this ignores the idea of dose-dependent transitions and the fact that as we really understand the systems approach, we recognize that that role of homeostasis is to bring us back to a normal state. And so the question of how we bring that into our assessment becomes important.

Endogenous chemicals with exogenous exposures presents some particular issues with regard to how we do our risk assessments. And the examples that I've listed here are just some of the ones that we had some reasonable data and some good knowledge on with regard to both the endogenous levels of these chemicals and exogenous exposures. And note that all of these are hazardous or potentially hazardous chemicals with regard to the list that the FDA has put out.

Just a few words about each of these: The case of ammonia, of course, is that this is a normal product of metabolism. We all carry levels of ammonia in our blood. At the same time we inhale ammonia from the environment, we exhale ammonia from our metabolism. The question of ammonia

irritating the upper respiratory tract is well established, but in order to be able to understand risk, one has to realize that those are exposures that are typically above the level that we're exhaling, although that is something that needs to be looked at carefully.

Acrolein is a constituent of various types of smoke. It is a reactive aldehyde. It's produced endogenously. It's typically found in human systems at levels that are measurable and can produce effects that we know have impacts on the cardiovascular system and occur basically because of both the peroxidation and amino acid metabolism and other sorts of things. And so the question of additional doses of acrolein to the biological system becomes the interesting issue as one thinks about risk of that particular chemical.

Formaldehyde and acetaldehyde, well-known examples of our aldehyde toxicities. In these particular cases there have been recent studies that have used new technologies in isotope detections that allow us to look at the difference between an endogenous and exogenous response to these aldehydes, and those particular studies have suggested the relative levels that one sees of the damage from these aldehydes endogenously as compared to exogenously.

Formaldehyde has been shown, in studies in animals now that are deficient in alcohol dehydrogenase and some of the repair pathways associated with Fanconi's anemia, to be a genotoxic agent under those kinds of circumstances that leads to bone marrow failure and other sorts of things that are traditionally looked at as a consequence of aldehyde exposure without any exogenous aldehyde exposures.

So these are issues that come up as we begin to think about these various chemicals which are highly reactive, and the question of where they're produced also becomes an issue.

The DNA protein cross-links associated with formaldehyde, for instance, are generated primarily from the degradation of histones, demethylation of histones right at the level of the DNA, and the question of the access of formaldehyde from outside to that that's produced endogenously becomes an issue for dose.

Carbon monoxide is one of three of the known gaseous transmitters that have been recognized over the last couple of decades as extremely important in terms of normal homeostasis in human biology. And so the question of where we see these tipping points or these changes in terms of a toxicologic response on top of a normal biological or homeostatic state is

something that becomes important for risk assessment.

So let's talk about complex mixtures testing for tobacco products. Again, some of the pros: They test the mixture of interest in comparable test systems. The constituent ratios are maintained over a dose range that is important for us, and it allows for us to look at biological responses to interactions that occur among those chemicals within the system.

And the downside is that they don't directly identify harmful constituents for us, if that's important, and they tend to be time consuming and expensive using traditional toxicology, as was already suggested. Trying to put these various mixtures together and test them, I think, will be talked about in a later talk today.

So the approach then is to begin to use some of these more modern techniques, particularly the omics techniques that will have impact on the full scheme of the system that we're talking about and, through the use of computational methods and bioinformatics, can lead us to some conclusions with regard to how the system is responding.

In terms of characterizing the transcriptome that comes about, Brody, in a discussion around tobacco smoke, used this

as an example that indicates that we need to be knowing, first of all, what we're talking about with regard to smoke, how it's smoked, what the amounts are, what the impact would be on the genetic system, as we've talked about. It's not just changes in genes or gene transcription. It has to do with the amount of message and microRNAs that are produced and epidemiologic changes. And this full transcriptome response is something that can allow us to look at and predict particular hazards and risks and perhaps even look at issues of prevention and diagnosis and treatment.

The way that one actually does this is through functional pathway analysis, as is shown here. The database that's generated from some of these high-throughput tests and transcriptomic approaches can go through everything from differential expression analysis to an identification of gene sets and down to the approach of looking at impacts on particular pathways that allow us to get to assessing the pathway significance.

So if we look at the mode-of-action analysis that we developed over the course of the '90s, it's very clear that that can be adapted to begin to take into account the kinds of issues that we're talking about with these more modern

techniques. And while we agree with the uncertainties and the data gaps that were identified, we also think this is going to help to guide future research to improve the understanding of this approach in health risk assessment.

As Lynne indicated, what's key to this is that this adverse outcome pathway is agnostic to particular chemicals. And so the idea is to really understand adverse outcome pathways in terms of biological responses and then overlay the dose to these targets that we're talking about from various types of assays and through the use of toxicokinetics. The idea then is to assure that we have an aggregate exposure pathway in terms of dose to targets and to think about this in terms of the results that would accrue from those particular exposures.

So I'd like to talk about a couple of recent studies in the last few minutes here. One of these is a study that came out in 2015 that is probably the first of the really systematic approaches to trying to use in vitro assessments, high-throughput assessments, in terms of risk assessment. It is listed here. And in that study, this particular diagram is used to illustrate the kind of attention to these various topics that would be required. The study itself looks at

quercetin, which is a flavonol that is in the diet but is also used in consumer products. And the assessment is to look at a lotion of this particular chemical, in terms of potential for risk, and it focuses on a DNA damage pathway mediated by p53 as a way to ask whether or not these particular levels of exposure perturb these pathways significantly at levels of consumer use. And so it makes for an interesting study that begins now, again, almost 10 years after the Academy report, to really focus on how these data can be used in risk assessment.

I want to give you two example studies that have just recently come out that have some relationship to the topic of our workshop today.

This particular study, the Shen et al. study that's described above, is one of the first studies to apply transcriptome profiles to determine if e-cigarette vapor alone poses a risk to human airway, and it evaluates human bronchial epithelial cells to e-vapor with and without nicotine and looks at cellular function and transcriptional activities. They compared the effects of exposure to that of mainstream smoke. But again, in this particular case it was not unexpected that e-vapor wouldn't elicit many of the cellular toxicity responses that can be observed in mainstream smoke, but in fact the

e-vapor did elicit discrete transcriptomic signatures with and without added nicotine.

And the question, of course, is whether or not the very small number of genes that were found to be differentially activated, but in fact quite a number of pathways that were significantly enriched in those particular studies, and the fact that this effect persisted, gives any indication of whether risk is involved in these sorts of things. And that will be the key question.

At this point, what we know is that cell cycle-associated functions and immune functions and metabolism of glycol constituents in e-vapor were actually impacted by this. So this is consistent with how we might think that these kinds of vapors would impact on a particular system.

The second example that I wanted to talk about again is a recent article that looked at the response of human bronchial epithelial cells to zinc. The idea there was to begin to look at this tipping point where the normal adaptive and cytotoxic responses are being determined. The analyses showed impacts on stress-signaling pathways, and there were 154 genes differentially expressed between the adaptive and the cytotoxic zinc concentrations, so there was clearly a difference once

that tipping point was hit.

If one then took those as a biomarker for that tipping point and compared it with other publicly available gene expression biosets in terms of the genes that were enhanced, the most highly significant biosets that these authors found actually originated from cigarette and tobacco smoke. And so the suggestion is that one can begin to use a chemical, explore an adverse outcome pathway, and then use those results to ask about other chemicals that similarly produce a gene expression like this.

So, in conclusion, then, I think we're making some progress in bringing 21st century toxicology into the risk assessment process, that the data that are being generated out of these high-throughput studies are of extremely good quality and tend to indicate opportunities for us.

The key to collecting valuable data from in vitro assays is talked about in the Adeleye study, and I think it's important to recognize that these need to be carefully designed much like our risk assessments need to be fit for purpose and with appropriate readouts.

Meaningful systems toxicology is required for us to have quantitative measures in the future to really understand these

homeostatic mechanisms and tipping points that I've talked about.

And additional work will be needed as we look at affecting cellular function and adverse outcomes that actually signify a human hazard potential.

But in the meantime, I think that these data consist of some comparative hazard characterization and certainly setting research and testing priorities for tobacco products.

Thanks.

(Applause.)

DR. DRESLER: Thank you.

Okay, our first break. It will be now for 15 minutes, so we'll come back at 10 after, okay? That gives us a few more minutes than 15, but 10 after. And I hope to start on time. Please remember that the food across that you see out there, or drink or coffee, does not belong to us. I'm sorry, but no. So if you want coffee, upstairs, okay? So go back down the hallway, up the stairs or elevator, and then to your right for coffee, okay? We'll see you back here in 15 minutes.

Thank you.

(Off the record at 9:53 a.m.)

(On the record at 10:13 a.m.)

DR. DRESLER: Come on in and grab your seats. And looking from the number of empty seats from now to when they went for it, they're probably in line for coffee, so I get that people will be coming back in.

So, Dr. Rice, they'll be coming back in. Maybe if I -- yes, a little bit more. Okay, so come on in. We're going on with Session 1, which is Hazard Characterization, and our next presenter is Overview of Chemical Mixtures Hazard and Risk Assessment, Dr. Glenn Rice from U.S. EPA.

DR. RICE: Thanks very much. Thanks for inviting me. Just the views. I'm from the U.S. EPA. The views that are expressed in this presentation are mine, and they don't necessarily represent those of the agency. I have no other conflicts of interest to acknowledge here.

So they asked me -- and I really do appreciate the invitation. They asked me to talk about two topics. One is just kind of briefly to describe hazard assessment from a chemical mixtures perspective, and I'll start with that, and that'll just be the first few slides. And then kind of as preview for what's coming up in subsequent sections, they asked that I talk about mixtures risk assessment methods. And Bill alluded to some in his talk, and it will probably kind of

dovetail pretty nicely, I think, with some of the things that he discussed.

So we'll talk about the component methods and whole mixture approaches, and then we'll wind up talking about the idea of sufficient similarity among chemical mixtures, which I think is an idea that I'm hoping will spark some interest and maybe get some wheels turning for this particular workshop.

So just as the three previous speakers, who all did an outstanding job of setting up my presentation, you know, hazard assessments, the idea of what is the potential for harm, you know, to determine if exposure to an environmental agent can actually cause an increase in incidence of adverse health effects in humans and whether that occurs in a human, again, is a really important concept.

Hazard characterization, which some of the speakers have referred to as well, typically applies to sort of an expansion on the hazard assessment to include the route, duration, timing of exposure, and sometimes even the mechanisms of toxicity, as both the two previous speakers noted. Typically, we're getting this information from toxicology and epidemiology data. So the timer's not running, which is fine.

(Laughter.)

DR. RICE: I just wanted to kind of point that out. If I'm speaking an inordinately long time, it's still where it was to begin with. Hey, all right. All right. So anyway, there it goes, it's going up now.

All right, so real quickly, so there are a couple different types of approaches that are used with mixtures. Sometimes we have information on the mixture of concern. Sometimes we have information on a similar mixture. Sometimes we'll have information on mixture fractions, which hasn't been talked about by the previous speakers. And sometimes we'll also have component information.

And all of these different types of information will generally be used for a mixture risk assessment, depending on whether they're available. So really it's availability of data that typically drives the information.

Where we have the choice, generally, we have increasing preference to information on the whole mixture of concern, and I'll talk a little bit about that from a hazard assessment perspective. And Bill also noted that in his talk.

When we're thinking about whole mixtures and whole mixtures toxicology, the real advantage, as Bill noted, is that you account for toxicity of unidentified compounds in the

mixture and for any interactions among the different mixture components. Obviously if we have an epidemiology study, we have increased relevance, as was noted by some of the previous speakers as well, because again we're dealing with the human population relevant exposure routes, potentially even relevant exposure levels.

Major limitations obviously are really a couple. One, as Bill noted, there's a lot of resource limitations with such studies. This idea of using whole mixtures can really be limited to just the mixture itself that was tested, unless those mixtures are judged similar, and we'll talk a little bit further about that towards the end of my talk. And finally, it doesn't really provide any information about specific information on interactions or individual components if you're just testing the whole mixture.

You can also evaluate different fractions. So sometimes it's difficult to evaluate a mixture in its entirety for a number of different technical reasons, often due to the chemistry of the mixture itself. For example, there are the risk assessments for petroleum hydrocarbons at Superfund sites that divide it into fractions, the aliphatic fractions and the aromatic fraction. That's work from the Total Petroleum

Hydrocarbons Working Group. The advantage here is it accounts for a lot of the unidentified materials in the various fractions and interactions among components.

There are obviously a number of limitations. You can't really look at interactions across fractions. You're assuming that the composition of the fractions are similar across mixtures, etc. You also can make assumptions when you combine risks associated with different fractions. So it does suffer from some of the similar limitations that we find when we look at components themselves.

If the components are known for mixtures, and a lot of times for some of the really complex mixtures, we don't know all the components, you can typically test these with a number of the different toxicological approaches that have been mentioned by some of the previous speakers. A lot of times we'll need the information on mode of action to actually assess whether the toxicities can actually be combined, and we'll talk a little bit about that in a few minutes.

Sometimes there are data to evaluate pair-wise interactions. There are a couple of databases that have some information on that.

But typically, one of the big limitations when we're

dealing with component data is we have to make a lot of assumptions regarding how the hazard that may be associated with an individual chemical can be kind of translated, if you will, into the hazard associated with the whole mixture.

So we'll start just to kind of follow the wishes of the people that invited me here to talk a little bit about component methods, and I'll talk primarily about the relative potency factor method, but we'll also touch on response addition in this slide.

So the relative potency factor method and the hazard index method, which Moiz Mumtaz will be talking about tomorrow, are both dose additive methods. So we're dealing with the sum of the doses to estimate some sort of a relative toxicity, and we assume a similarity of toxic action among the components, specifically if the chemicals elicit or would be expected to elicit a common biological response. And just to put it in terms of an AOP, each chemical would elicit a common key event within the agnostic adverse outcome pathway, where the doses would be expected to add or would actually add.

There's also another approach out there. This is called simple dissimilar action, and we use response addition. This is an approach that's used in a lot of EPA Superfund sites

where they'll sum the probabilistic risk of carcinogens. This assumes both a toxicological and a statistical independence.

So dose addition: I just want to get a little bit more into this. We were kind of asked to kind of provide some specific examples. So this is the dose addition formula for a relative potency factor. We see the risk, you know, for the mixture response R_m here. We use a dose-response curve from one of the chemicals, one of the components, and then we kind of use that with a combination of the chemical itself and then a second chemical. And you can expand this to other chemicals where the potency is influenced by this factor t. And for this particular case, it's the potency of the two chemicals relative to each other. So we're looking at the effect of dose, the ED here, down here in the formula of Chemical 1 over that of Chemical 2 to estimate this factor t. And I'll show this in the example that I'm about to use from the organophosphate pesticides.

So this is a group of pesticides that EPA regulates as a mixture. They started work on this in the late 1990s. EPA selected methamidophos as the index chemical. So this is the chemical to which all the other chemicals are going to be -- the toxicity of all the other chemicals is going to be

evaluated to estimate their toxicity relatively, so it's a relative potency factor approach.

So the reason they chose methamidophos is that the dose-response curves are similar across routes, across compartments, across different sexes. They had a lot of high-quality data from both oral, dermal, and inhalation routes. And in this plot, you can just see there were three different rat studies that looked at decreases in acetylcholinesterase activity with increasing dietary dose.

So when you're actually applying this method, you have to assess the method -- you have to assess the toxicity for each member relative to some index chemical, and typically we prefer benchmark dose approaches to no observed effect levels or lowest observed adverse effect levels. And typically, that BMD will represent a common response level of approximately 10%. And then, as I noted previously, each member is scaled on potency relative to an index chemical as related in the formula below.

Just to kind of show you what these relative potency factors, these *t* values, actually look like, you can actually see the index chemical. Methamidophos is one. This is done on a log scale. You can see that some of these components are

actually more toxic than the index chemical, and a lot of them are a lesser component. But you've got sort of the idea of increasing relative potency as we go from left to right.

I want to talk about another type of approach, which is actually information on the mixture of concern. So the idea here is that you can actually develop a reference dose, which is what EPA uses for non-cancer assessment just as an estimate of the daily exposure to a human population that's likely to be without an appreciable risk over the course of a lifetime exposure. And the idea here is that you can look at a whole mixture toxicology or epidemiology study, evaluate no observed effect level or a lowest observed effect level and/or a benchmark dose approach, and then divide that by the specific uncertainty factors the EPA uses normally in their development of reference doses and actually develop a reference dose for an entire mixture.

There's an example of this on EPA's integrated risk information system, which is the polychlorinated biphenyls. And here what they did is they had a study of one particular type of PCB, which is the Aroclor 1016 that looked -- the health effect of interest was a reduced birth weight of monkeys in reproductive studies, and they divided that by four

different uncertainty factors to estimate a reference dose that you can actually find on IRIS.

Notice that this particular reference dose, the confidence is actually medium, and that's because mixtures of PCB in the environment do not match the congener profiles that are actually found in Aroclor 1016. So this idea of whether the mixture that's been tested and for which a reference dose has been developed is actually applicable to environmental mixtures of PCBs is kind of where I'm going next in the talk, so kind of just to set that up a little bit.

So now we're going to deal with the idea of similar mixtures and just approaches for how we would actually begin to think about similar mixtures.

So this basically comes from EPA's 2000 chemical mixtures guidance on sufficient similarity, and the idea here is that if toxicity data are available for a chemical mixture of concern, you obviously base the information on that mixture. If they're not, then you can use that same information to base it on a sufficiently similar mixture.

Mixtures are sufficiently similar to one another when the components are not very different and when they're in about the same proportions. So that's exactly --

(Laughter.)

DR. RICE: Yeah, that was kind of where we kind of wound up with it in 2000, and we've begun to do some research in different places to start to look back at that. Not being done. We've been doing research to kind of evaluate how to handle that.

You also want to see few differences in environmental fate, which was noted by the PCBs example, uptake bioavailability, and pharmacokinetics. But the idea here is that the toxicologic consequences of exposures to two different mixtures are nearly identical and with relatively few differences in the toxicological effects.

So what kind of information do we want to have? Obviously, we want to have relevant chemistry data for both the tested mixture as well as the mixture of concern, which in our case is in the environment. We want to know the major chemicals, chemical classes, the relevant proportions of the chemicals. We also might want to know some information on the characteristics of the unidentified fractions, so maybe a set of the chemicals that are halogenated, that kind of thing.

We also want to have relevant toxicity data for both the tested mixture and the mixture of concern, because we want to

be able to make some reasonable comparisons. We'll have to have dose-response data for the tested whole mixture; we'll need to have that. And then we might have some toxicity test data for either a precursor effect, something like was indicated by Dr. Farland, for the tested mixture or the mixture of concern.

We also might want to have some toxicity information on key components or component groups, which might be useful either in a hazard assessment or to kind of further confirm that we're actually dealing with mixtures that are relatively similar from a toxicological perspective. This information can also be used to help explain relationships among mixture components.

So some of the useful characteristics to evaluate sufficient similarities: Prior to the evaluation itself, we want to establish criteria for judging whether mixtures differ or not. We want to have a test that's transparent and practical and very easy to understand and easy to apply, that's applicable to different measures of chemical composition and different types of toxicity and consistent results, you know, regardless of who the analyst is. So, again, this idea of transparency and replicability being two really key

characteristics for the approach that we're talking about here.

So here's a quick example that I want to kind of share with you. This is a paper by McDonald et al. from 2004, and they looked at engine exhaust, and they specifically looked at exhaust from different types of engines, and one of the goals was to evaluate the similarity among these very complex whole mixtures. They had relatively little component toxicity information that they were going to use for their assessment, but they did have a fair amount of toxicity information and chemical information for the whole mixtures.

They used a principal components analysis approach as well as multivariate regression, and I'll explain the principal components analysis briefly in a subsequent slide. And they investigated statistical relationships among exhaust samples that were collected from gasoline- and diesel-powered engines, and they also then evaluated those against rat lung toxicity.

So they had these whole mixtures that were collected from gas cars that were operating at a room temperature as well as those that were operating in the cold at about 30 degrees Fahrenheit. You can see there are a number of different types of gasoline exhaust samples that they collected, as well as a number of different types of diesel exhaust samples that they

collected.

Okay. They used 11 different toxicity tests in their analysis. They looked at inflammation potency estimates based on number of different aspects of lung inflammation, and they looked at cytotoxicity. They looked at parenchymal cells and general toxicity for the lung. So they looked at a number of different aspects of lung toxicology.

Just to step back a little bit, principal component analysis is used here in a cluster analysis perspective, and it captures the variance of the dataset while reducing its dimensionality. And then each variable here is a transformed linear combination of original variables and the two that are represented here, Principal Component Analysis 1 and 2, are uncorrelated.

The blue lines represent two consecutive principal components that are orthogonal to each other. Principal Component 1 would explain most of the variance in the dataset, and Principal Component 2 then would explain the next level of variance in the data.

So here's the score plot that they developed using their 11 different lung toxicity assays for the different groups, and you kind of see that these actually kind of group -- even

though they're very different types of engines, different types of exhaust, you can begin to see that they would group into a couple different -- three different groups specifically here. They're just sort of highlighted for illustrative purposes. You could also use, obviously, statistical analyses to actually evaluate the differences in these groups. But you can begin to see that these groups are actually quite different, and if you're using sufficient similarity, you would want to have the dose-response assessment for one of these mixtures, and maybe you could assume that the others are sufficiently similar in these three different groups.

So principal components are transformed variables that consist of kind of real-world variables combined in different ways. For this particular example, the choice of toxicology tests would depend on the health effects that we're really concerned with, if we're actually concerned with a mixture.

For this particular case, they were very concerned about lung toxicity, so that's why they looked at the 11 different toxicity tests for the lung data. To apply sufficient similarity methods for those samples, you would need then to develop dose-response functions to the mixtures as a whole that could be reasonably used and inferred across the broader sets

of mixtures.

Okay. So just in conclusion -- I'm going to wrap up a few minutes earlier -- there are multiple ways to evaluate hazards posed by a mixture. You can test whole mixtures. You could look at fractions of components. Obviously, risk assessors would prefer whole mixture methods.

We talked about two component methods and just the different assumptions that underlie each of those, and then also the idea of setting a health reference value for a whole mixture base for the whole mixture itself. The difficulty with that is how applicable is that particular reference value or cancer dose-response assessment to other mixtures that are similar.

So given the resources that are necessary for evaluating dose response of environmental mixtures and the many different mixtures that we're going to encounter not only in the environment but with all the different types of tobacco products that are used, sufficient similarity, I think, may be a potentially very useful approach for estimating a mixture's risks.

And with that, I'd like to just to kind of tie it into Bill's idea, the idea of using omics data, and again the idea

that you can begin to look at a number of precursor effects with AOPs to further evaluate kind of upstream effects, if you will, before we actually have to deal with cancers and things like that that might be along that pathway of toxicity to an ultimate endpoint like that.

A number of different folks who I drew materials from or have worked with in the past, I'd like to just acknowledge them in this particular slide, including Dr. Mumtaz and Dr. Hertzberg, who will be speaking later.

And finally just to say thanks very much, and we'll take questions at the end.

(Applause.)

DR. DRESLER: Thank you. So the timer is controlled in the back. I just keep an eye on it, so you were good. Thank you so very much. Thank you.

Okay, so our next presenter is Dr. Kate Guyton, and she is from IARC, or the International Agency for Research on Cancer, and she will be speaking on Cancer Hazard Identification in the IARC Monographs Programme. So she will be speaking remotely, and we're going to see technology work.

(Laughter.)

DR. DRESLER: Now, don't laugh; you all do that. And I

can tell a variation in the microphone, so it works.

Dr. Guyton?

DR. GUYTON: Hello, this is Kate Guyton. Can you hear me?

DR. DRESLER: Yes, we can hear you quite well. So I did introduce you and the title of your talk. Your slides are up. Please go for it.

DR. GUYTON: Great. Well, good afternoon or good morning, everyone. Bonjour. I am Kate Guyton. I'm the senior toxicologist in the IARC Monograph Programme at the International Agency for Research on Cancer, which is in Lyon, France, and IARC is part of the World Health Organization.

So the next slide.

(Pause.)

DR. GUYTON: There's a little bit of delay.

DR. DRESLER: It's a big ocean.

(Laughter.)

DR. GUYTON: I can still see just the first slide there.

DR. DRESLER: So we have up in the room the conflict of interest statement. Is that the one that you're looking at?

DR. GUYTON: Okay. Yeah.

DR. DRESLER: Okay.

DR. GUYTON: So I just wanted to begin by declaring no

financial interests, and I also don't have any current or past employment, research support, or other professional relationships with an entity involved in tobacco or tobacco products. And for those of you who have been on an IARC working group or participated in any WHO meeting, this is the type of disclosure that you would be familiar with.

So, on the next slide, I'd just like to give you an overview of my presentation. I'll start with just some background on the IARC Monograph Programme, focusing on our evaluations of tobacco and tobacco components and hopefully complement some of the other great speakers by really zeroing in on our evaluation of epidemiology evidence, but also some of our recent progress within evaluation of mechanistic data with regard to the published literature as well as the Tox21 data.

So, on the next slide, -- I'm just seeing a little bit of a delay for the slides, so I'm not sure. I'm still on the one that's a presentation overview. But the next one, which really concerns the global burden of cancer at IARC, from our colleagues in the GLOBOCAN program, they predict that there will be nearly 20 million new cases of cancer by 2025 compared to about 14 million in 2012, and the majority of this increase is expected in low- to middle-income countries. Now, this is a

challenge, but we don't expect to be able to treat our way out of this problem. We do believe that prevention is the single most effective response, and the IARC monographs play an important role in this by identifying the causes of cancer, and we have a companion program that addresses what prevents cancer.

And on the next slide I'd like to just give you an overview of some of the work that we've done in the monograph and in the handbook.

DEREK: Dr. Guyton, this is Derek with the AV staff --

DR. GUYTON: Yes?

DEREK: -- here at the meeting. I just want to point out to you that we are advancing the slides for you, but there will be about a 10-second delay if you're watching along on the webcast.

DR. GUYTON: Okay.

DEREK: So when you call for a next slide, if you could just trust that we're advancing in the room for you --

DR. GUYTON: Okay.

DEREK: -- you can follow along on your own, okay?

DR. GUYTON: All right, I'll do my best.

DEREK: Thank you.

DR. GUYTON: And thank you. Thank you so much for that, I appreciate it.

So the monographs which are shown on the left, we've developed a number of monographs on tobacco-related issues since the 1980s to the 2000s, and on the right-hand slide -- you might have to hit next a couple of times -- we also have a number of handbooks addressing issues as well. And also there was a working group that IARC in the Tobacco Free Initiative -- through the Tobacco Free Initiative participated in a WHO group on tobacco product regulation. So these were some of the books that we have available and highlight some of our past product progress in this area.

So, on the next slide, you might wonder how the IARC monograph evaluations are conducted, and if you hit next a couple of times, you could say, well, if you had a bunch of data, how do you turn it into one of these orange-covered books that are known as the monographs? And all of this is shown in our preamble, which is available online. It's a relatively short document of about 25 pages, and it covers everything from procedural guidelines for how we select participants to how we manage conflicts of interest, engage with stakeholders, and conduct our meetings. But it also gives the criteria for

review of the human, animal, and mechanistic evidence as well as the decision process for the overall evaluations. And I'm going to go into some of these, some of the details of this in the next few slides.

So, on the next slide, the first question is really, who does the evaluation? I work for IARC, and I don't do the evaluations. These are done on the next -- shown next. It's really done by a working group of independent scientists who don't have a conflict of interest, and they're the ones who review the science and develop the evaluations. Now, there's also the IARC secretariat, which is me, and we could do things like coordinating all aspects of the evaluation.

Now, if you hit the next, you'll see that there are other types of scientists who can attend a meeting but do not write the reviews or contribute to the evaluations, and they fall into three categories, which include invited specialists who are scientists with relevant knowledge but have a competing interest, we also have representatives of government and health agencies, and finally observers, which are scientists with a competing interest and are there to observe but not to influence the outcomes.

So the next slide covers what type of evidence we

consider, and if you hit next again, you'll see that this is really three main lines of evidence: cancer in humans, cancer in animals, and also the mechanisms. And this is what feeds the overall evaluation.

Now, if you hit next again, we also consider the exposure data, but that is really kind of a separate issue that doesn't weigh into the evaluation per se, so I'm not going to discuss that further.

Now, the next slide really gives an overview of the evaluation process, and this is a two-step process. The first component has to deal with categorizing each line of evidence separately. So for cancer in humans and cancer in experimental animals, this involves determining if there's sufficient, limited, or inadequate evidence of carcinogenicity or evidence suggesting a lack of carcinogenicity. The mechanistic and other data are judged to be weak, moderate, or strong, and also whether this is happening in humans, can happen in humans.

So the second step, if you hit next again, is to bring these together in the overall evaluation. And what's listed here are the different groups that we do have, and the numbers following that are the number of agents that we have categorized into each of these groups in the history of the

program.

So with almost 1,000 agents evaluated, you'll see that most of these are falling into Group 3, which is not classifiable as to the carcinogenicity to humans. We also have put only one agent into Group 4, which is probably not carcinogenic to humans, but as indicated in our preamble, we evaluate agents that have some evidence or a suspicion of carcinogenicity, so perhaps this isn't surprising.

Now, other types of agents have been evaluated mostly into Group 2B but also into higher levels of evidence, and we're going to go in the next slide in some more detail about how this is done.

So as I said, I'm going to focus a little bit on the epidemiology. That's a topic that not a lot of others have covered here today. And if you go to the next slide, and then I think it's just going to be two more clicks to get into this issue of cancer in humans, and I've indicated the place in the preamble where you can read more about this, and this is going to discuss the causal framework of Hill and other considerations that are taken into account.

And the categories of evidence are really listed here. Now, to get sufficient evidence, this means that a causal

relationship has been established. This is based on studies in humans and indicates that chance, bias, and confounding could be ruled out with reasonable confidence.

Now, limited evidence is applied when there is a positive association and if a causal interpretation is credible; however, chance, bias and confounding could not be ruled out with reasonable confidence.

Also, there is inadequate evidence as another possibility, and evidence suggesting lack of carcinogenicity.

So in the next slide I'm going to show you how these overall evaluations are determined. So this is really according to this matrix that I'm showing here, where you bring together, first, the evidence in humans and evidence in experimental animals, and I'm going to show you some examples to really illustrate how this is done.

So, on the next slide, as I said, for the agents that are in Group 1, this is typically driven by sufficient evidence of cancer in humans, and examples of this include tobacco smoking, secondhand tobacco smoke, smokeless tobacco, as well as betel quid.

So another possibility is to have, shown on the next slide, limited evidence in humans. Now, if you just have

limited evidence in humans, this would go to Group 2B, and an example of this is styrene.

Now, on the next slide, another possibility is if you had inadequate evidence in humans but you had sufficient evidence in experimental animals, and this would also go to Group 2B, and examples of this are things like isoprene and catechol. And this is the case for most of the agents that are categorized in Group 2B.

Now, as shown on the next slide -- I'm up to No. 15 -- another possibility is that you would have limited evidence in humans and sufficient evidence in experimental animals. So this is really a combination of the two possibilities I just showed you, and this could lead to a higher categorization into Group 2A. An example is inorganic lead.

So, on the next slide, finally, the other possibility using this data. If the evidence in humans is inadequate, and evidence in the experimental animals is limited or inadequate, then you would end up in Group 3, and these are things like mercury and phenol. And as I mentioned, this is where over half of the agents that we evaluate would end up.

So, on the next slide, I just want to introduce the idea that mechanistic data can be pivotal when the human data are

not sufficient. So now I'm going to take you through a few examples of how the mechanistic data can play a major role.

The first example is if we had sufficient evidence in experimental animals and inadequate evidence in humans, which would, as I showed you earlier, normally result in a Group 2B categorization. However, if you have strong evidence that the mechanism in animals does not operate in humans, and several of the earlier speakers alluded to these types of examples like saccharin and *d*-Limonene, then that would also result in a downgrade to Group 3.

So, on the next slide, you could imagine a similar situation where you have the sufficient evidence in experimental animals, but you also had strong evidence of a mechanism that also operates in humans. And in this instance, you could actually raise the evaluation to Group 2A, and there are some examples of this, like dibenz[a,h]anthracene and so forth, that have been moved up in the classification or what we call an upgrade.

So, on the next slide, I just also want to raise the possibility that if you have that limited evidence in humans and sufficient evidence in experimental animals, which would normally put you in Group 2A, then as shown in the next slide,

that this can actually -- if you had strong supporting evidence in exposed humans, that you could actually raise the classification to Group 1, and there are some examples of this: ethylene oxide, NNN, NNK, benzo[a]pyrene. And I'll come back to these later. And this has been done for about a dozen agents. It does really require that evidence in exposed humans.

So hopefully that has now given you an overview of how these evaluations are done. All of this is really described in detail in the preamble, which gives, as I've mentioned, more detail about how the procedures and the evaluations occur.

Hopefully we're caught up now to Slide 20. So now I'm just going to switch gears and talk to you a little bit more about some of the classifications of tobacco, and that's what's shown here on Slide 20. And as I mentioned before, tobacco smoking, smokeless tobacco, secondhand tobacco smoke, and betel quid are all classified in Group 1.

And on the next you'll see that this is really based on sufficient evidence in humans for carcinogenicity, and it covers tobacco smoking but also parental smoking and, as I mentioned, smokeless tobacco, secondhand smoke, and betel quid.

Now, on the next, I just thought this would be a good time

to emphasize that the higher classifications refer to strength of scientific evidence. It's really about causality. Does A cause B? It doesn't reflect the level of carcinogenic risk or who is experiencing this, right? And this is why, for example, tobacco smoke, tobacco smoking, and secondhand tobacco smoke are in the same group, but they cause different kinds of cancers, they would have a different dose response, and people might want to take different actions as a result of these classifications. So this is something very important to keep in mind.

So speaking of the types of cancers, let's go to the next slide. And I just want to point out that really since the '50s, I think it's been known that smoking causes lung cancer. But when IARC evaluated this evidence back in the '80s, there were also some other cancer sites that were evaluated, and these are shown in red. And through a series of subsequent evaluations, the number of cancer sites has been expanded significantly, and these are shown here.

And on the next slide, it's just to now talk about the types of cancers that are associated with different forms of tobacco, just listing the cancer target sites here on the left, some of the ones that are associated with tobacco smoking based

on sufficient evidence for carcinogenicity in humans. There's also evidence for secondhand smoke, smokeless tobacco, and betel quid of different types of cancers.

So I think it's important to bear this in mind, that there can be different cancer sites depending on -- as one of the earlier speakers mentioned, we have the portal-of-entry considerations, but we also have some knowledge that some of these tobacco carcinogens are able to move around the body following the exposure.

So speaking of the tobacco smoke carcinogen, going to the next slide I just wanted to point out four agents that IARC has classified in Group 2B or higher. These comprise a number of different chemical classes, including things like aromatic amines and aldehydes as well as metals and PAHs. So this is really only a small subset of the chemicals that have been identified in the mixture. But among these we do know that there are -- several of these have already been classified by IARC.

And I thought it might be of interest, as shown in the next slide, to talk about -- to show you the tobacco smoke carcinogens that would have limited or sufficient evidence of carcinogenicity in humans, and this includes some of the

aromatic amines, aldehydes, some volatiles like benzene, 1,3-butadiene, as well as several of the metals. For others, then, such as lead, we do have limited evidence in humans.

And then I also wanted to point out, as I said before, there are several of these where the evidence in humans is limited, but these are classified into Group 1 based on consideration of the mechanistic data and specifically evidence in exposed humans that is consistent with carcinogenicity.

So now I'm going to switch gears a little bit and just kind of take these three examples forward and talk a little bit about some of our developments in the area of mechanistic data and how we've looked at some of these Group 1 carcinogens and how this has really been the basis of our effort in this area.

So, on the next slide, there's the agents I just mentioned that have been classified into Group 1. That's essentially based on their genotoxicity, so these are like genotoxic effects, cytogenic effects, adduct mutations and this kind of thing. And this has really been happening since the '90s.

But if you click next, you'll see that there are other agents that are also in Group 1, like dioxin, that have different effects. They aren't genotoxins; they're really binding receptors or doing other things that are quite

distinct.

So in the next slide I just now want to talk about the Volume 100 review that was taken on by the IARC Monograph Programme under the leadership of Vince Cogliano when he was here, and this reviewed all of the known human carcinogens, all the agents that are in Group 1. And what came out of this meeting in terms of the mechanistic data, if you hit next, is really that different human carcinogens may operate through distinct mechanisms -- I think I just showed you that -- but also that many human carcinogens are acting through multiple mechanisms. And a third challenge is that there's really no broadly accepted systematic method for assembling and evaluating these mechanistic data.

So during this review, based on this Volume 100 review and in subsequent workshops, what came out of a lot of expert discussion was what's shown on the next slide, which are these, what we call the key characteristics of human carcinogens.

And if you hit next, you can see that, essentially, established human carcinogens are commonly exhibiting one or more of these characteristics. And if you think back to the actual carcinogens, I mean, these are things that some of them do, being electrophilic, exerting genotoxicity, inducing

oxidative stress, chronic inflammation, etc. But if you think about the whole range of human carcinogens, including viruses and fibers and other types of chemicals, you can see how this list would apply really to these established human carcinogens of many varieties.

And the publication is shown here on the bottom of the slide, and I encourage you to read this paper. It's really the brainchild of Martyn Smith, who's at the University of Berkeley, the University of California at Berkeley, who put together this list and to kind of bring forward this idea that we could use this information on these key characteristics really as kind of a framework to think about how we think about mechanisms, as they really provide evidence of carcinogenicity. It's really kind of extending the causal framework that IARC uses into the mechanistic area. And they can also help in interpreting the relevance and importance of findings of cancer in animals and in humans.

So, at IARC, we're really very interested in applying these to kind of develop a systematic approach to evaluating mechanistic data, which I think has really been a challenge, and a lot of people say, well, you know, this is absolutely impossible, there are too many studies, there are too many

mechanisms, the data are just much too diverse, and it's impossible.

So I heard that a number of times, but I just want to remind you, as shown on the next slide, that there has been a lot of progress in this, and I just want to -- in this area and want to just highlight this publication. This is a project that Mary Kushman led. I believe I see her sitting in the audience, but I want to give her a lot of credit for kind of developing a practical approach, saying, hey, you know, we can take this and take even a very, very complex mechanistic database and start to make progress in how we systematically identify mechanistic data and force them out and summarize them.

And the next slide is really kind of taking this forward with this approach and learning from that experience as well as trying to apply the key characteristic approach, which really could be applicable regardless of whatever the exposure is.

And if you read the paper, you'll see that what was done was really to develop targeted searches for each of the characteristics and then using the system called HAWC, which is Health Assessment Workspace Collaborative. But you can also do this -- you can do this in PubMed, you can do it with a pencil

and paper; it doesn't really matter how you do it. But essentially, you can do systematic searches, and you can really then organize those results according to the characteristics, species, and other topics. And, of course, we do searches on things like toxicokinetics and looking for susceptibility, etc. But the key characteristics are really kind of now the central component of what we're doing at IARC in terms of identifying, organizing, and evaluating the literature information on mechanisms.

So, on the next slide, I just wanted to point out that we've heard the challenge from Dr. Farland about moving forward in evaluating in vitro data and moving forward into the Tox21 approaches, and I want to just share with you some of our progress in this area.

And so if you think about the classes that are available there, which is over 800, and then you think, well, we have these 10 key characteristics, well, it sounds like a very simple math problem. But as it turned out, really, when we decided to just go into this dataset and to just start categorizing these assays using the prism of the 10 key characteristics to say, well, really, how informative are they about carcinogenesis, we did find that there are several

characteristics -- and you'll have to hit next a couple of times to populate the slide -- that really we don't have a lot of assays, I think, for genotoxicity, which is less of a concern because we already have an in vitro battery for that and many chemicals that have been run through it. But for a lot of these other key characteristics, there aren't a lot of assays. And this is a paper that we have under development, but we've also published some of it in recent monographs, if you want to take a look.

And what that's going to show you on the next slide is really how we can use these data to really answer key questions. And the questions that we see, which are shown next, is really what we're interested in, in these kind of -- and the advantages that these big databases offer is really a comparative approach. You can compare your chemical to every other chemical that's in there.

And from our perspective, we've done some comparisons also by key characteristics. You know, how does the evidence compare across key characteristics and also within them? And also if there are data available on metabolites, then this can be another interesting question you can explore.

And then if you hit next a couple of times to populate

this slide, you'll see that we applied this ToxPi methodology that was developed by David Reif when he was at EPA in collaboration with other EPA colleagues, and it's just a methodology that helps you visualize a lot of diverse information and to really aggregate that in a way that allows you to make comparisons across and within chemicals. And I'm just showing here that you can build these ToxPis to reflect evidence on all of the key characteristics, or you can drill down on any one key characteristic. And as I said, it's a comparison.

So the approach we took was to do this with respect to the top inducers from the database to give some perspective on the activity we are seeing. And I'm showing the results for malathion and malaoxon, its metabolites, and you can find more about this online if you go to our website.

So just in closing, I just want to show you a couple more slides. So the next is really what some people like in terms of how we summarize the evidence on key characteristics. I think it's possible now to say to take each one and to really answer this question, is the data -- is it weak, moderate, or strong? There are some people who like to see things visually, and this is a slide that I borrowed from Martyn Smith, and it's

showing, well, can you use these to really construct an adverse outcome pathway? And here you have benzene exposure going to leukemia.

But if you go to the next slide, you can see that it really starts to get -- when you consider all of these key characteristics, it gets a little bit more complicated, and I think Martyn phrased it as an adverse event outcome network with different arrows going in different directions sometimes, depending on the types of events that are captured there. And that's published in our paper that I referenced earlier.

So, on the next slide, just to summarize, tobacco products are carcinogenic to humans -- that's in Group 1 -- like diversity of tobacco components are carcinogenic in animals and in humans.

And I talked to you about the key characteristics of human carcinogens, including the tobacco carcinogens, can provide a basis for an objective approach to identifying and evaluating the mechanistic evidence. They can allow the development of credible adverse outcome networks based on systematic review, and they also lay important groundwork for future evaluations where such data can fill in important gaps in the evidence of carcinogenicity.

And in the next slide I'd like to acknowledge my team here at IARC and also our funders who are listed here.

And finally, on the last slide I'd like to thank you very much. Merci beaucoup. And I have listed here our website where you can find more information. So thank you very much for your attention, and I want to thank whoever's in the room who's taking care of my slides; I really appreciate it. And I look forward to the panel discussion.

(Applause.)

DR. DRESLER: So now our next portion is to have the speakers that are in the room come on up. You're getting nametags up there, so come on up to the front if you would, please. Dr. Guyton, I assume you're going to stay on the phone, so if we have questions for you.

If there are questions in the room, kind of raise your hand, and you'll get some cards. People have written cards already, so just raise your hand, and you can get cards to write them down. When you've written them, just hold them up; somebody will come through and pick up the cards, and then you can -- they'll bring the cards up for me to ask the questions.

We have approximately 50 minutes for this, so if I could ask this to go on because -- 25 minutes, okay. So if you'll

put the timer on because I keep a watch on that because we don't want to miss lunch. Okay, so you have your cards if you have questions. If you're online, please send in your questions.

The other thing, too, is that if you all registered on workshop.CTPOS@fda.hhs.gov, give us a couple of days. If you want the slides, you can e-mail that and ask for the slides. All the time that question comes up; people want the slides. So please don't do it to us in the middle of the meeting, but if you e-mail it, we'll work on that in the next few days, okay? So that's workshop.CTPOS@fda.hhs.gov.

And then, also, we have a panelist, Dr. Julia Hoeng, who is joining our panel. So if you would like to go ahead and introduce yourself. And welcome.

DR. HOENG: Yeah, thank you very much for having me on your panel. So my name is Dr. Julia Hoeng. I work actually for Philip Morris International, where we have been setting up a systems toxicology approach for comparative risk assessment of our NRT products, and I hope to share with you maybe some of the insights and also add some value in the conversations.

DR. DRESLER: Thank you.

The other thing I wanted to mention, too -- Dr. Guyton,

you're on the phone. Yes, Dr. Mary Kushman is in the office, and we owe her a lot of gratitude because she has been working with us in the Office of Science on what you were presenting, so she's been helping us in the Office of Science. So thank you, Dr. Kushman.

Okay, so cards are coming in. Sometimes I feel like a game show host with these questions, so this is the fun part.

Okay. These are difficult topics, you know? The presentations were superb, but as you all are aware, these are quite complex. So let me start out with a hard question, actually, what other criteria to consider for identifying expected or unexpected adverse events in the use of tobacco. So this will go for anyone on the panel, but what are the criteria to consider for identifying expected or unexpected adverse events in the use of tobacco?

And I know you all want to jump on it at the same time, so if you push that red button that's in front of you, then that will turn your microphone on. And then, also, if you'll turn it back off because there gets to be sound competition for the recording and people online.

Dr. Farland, I see you thinking about pushing that button.

DR. FARLAND: So I'll jump in to start. Thanks. It seems

to me that, you know, we'll continue to use our sort of tried and true toxicologic criteria as well as the Hill criteria that have been established for epidemiology studies to really think about these criteria. I don't think that this is a problem that is different than most others that we deal with when we're dealing with complex mixtures.

So the criteria really have to do with the consistency of the data and the applicability to human risk and the ability to demonstrate, at some point, that the exposures are actually in the range of concern. So I'll stop at that point.

DR. DRESLER: Anyone else want to jump in? Okay. Lynne, please.

DR. HABER: I think that was very well said. I guess just one thing I wanted to add, since Dr. Guyton referred to the seminal paper on the key characteristics of cancer, that in evaluating adverse effects, we need to -- and particularly in predicting cancer, that we need to distinguish between endpoints that are hypothesis generation or suggestive versus things that are definitive. So we certainly know that a positive Ames test does not necessarily mean that a chemical is causing cancer or that it's causing cancer via mutagenic mode of action. So, similarly, we know many chemicals that cause

inflammation that do not cause cancer. We know many chemicals that cause cytotoxicity that do not cause cancer.

And, of course, this is one of the very challenging issues in mode of action evaluation, so we still need to be including that whole mode of action evaluation in evaluating the potential for an adverse outcome.

DR. HOENG: I think all the points mentioned were very good. Maybe we could just also add that when you actually work with novel type of tobacco products, you may want to reevaluate the applicability of the test systems that you might have set up for conventional cigarettes, because a lot of the novel products, you know, these are different types of errors, also. I think fit-for-purpose of your test system is also an important aspect to be considered.

DR. DRESLER: What factors may be considered to evaluate the relevance of in vitro results to in vivo effects? So what factors may be considered to evaluate the relevance of in vitro results to in vivo effects? Who said they were going to be easy questions?

(Laughter.)

DR. FARLAND: So I think to address the question, we really have to think about this from the standpoint of how we

have evaluated test systems in the past, and a lot of this has to do with the applicability to relevance to humans.

So on the one hand, one can think about this from the standpoint of the animal test systems as a surrogate for humans and the applicability to an outcome as being assumed to be reasonably interpreted as a potential for human hazard, as opposed to thinking about the animal as a separate system and contrast that with the idea of sort of understanding the biology and first principles and being able to look at the results from the standpoint of whether or not they are on the continuum to an adverse outcome in terms of first principles, and then determining whether or not those are likely to occur in a human population that's exposed. So I think trying to put those two things in balance really are -- is a way for us to think about the answer to that question.

DR. DRESLER: Okay. Dr. Hoeng.

DR. HOENG: Yeah, I might jump in here as well because we've given this a lot of thought also with the idea to reduce the number of animal experimentation. Clearly, there are opportunities for what we call mechanistic disease assays. So in cardiovascular, for example, you could develop a cell adhesion assay, test your product, investigate cell adhesion

on, actually, primary human cells. Then you could leverage a rodent model and maybe start to actually look at the cardiovascular endpoint with the same type of product. And ultimately, in your adverse outcome pathway, you could identify biomarkers as surrogates and measure those in short-term clinical studies of soluble ICAM.

So there are opportunities; actually, that's why I very much like the adverse outcome pathway framework. While it is a framework, it enables you to conceptualize the initiating event to the adverse outcome, and it also allows you to actually aggregate different experimental systems.

So they don't all have to be your classical OECD type of assays, but they're actually opened that you can incorporate modern mechanistic assays, air-liquid interface assays, mouse model data. And you rank that, always the weight-of-evidence approach, but ultimately you sort of define a framework where you go from an initiating event to an adverse outcome. It's hard to do, but I think we're getting better at it.

DR. HABER: So both excellent comments. The other thing I just wanted to add is, of course, the importance of anchoring what we're seeing in in vitro assays to in vivo data, our understanding of the biology, as that was noted, and that one

of the big challenges is that often we're trying to link that in vitro data with in vivo animal assays and the rodent data, and we need to be careful, that the rodent data don't perfectly predict the effects seen in humans and particularly, they don't perfectly predict the effects seen by target organ basis. So we would need to really be looking at the biology to help us understand things.

Also this idea of air-liquid interface has been mentioned a couple of times, and I just wanted to expand on that, that as we're looking at validating these in vitro assays, that there's a whole continuum ranging from the transcriptomics to looking at the specific endpoints, as was mentioned, for ToxCast, which has the advantage of looking at a number of key events in mechanistic studies but also, as was noted, has a number of gaps for -- it only looks at specific steps in the pathways. And then there are these organ-on-a-chip sorts of approaches, which aren't a high-throughput approach but are still very powerful and can look in vitro much more efficiently than in vivo. And among those there are these air-liquid interfaces where you're actually looking at human epithelial cells that include many of the characteristics of the complex cell mixture in the human lung, including the mucus, cilia, etc.

So these are amazing test systems; some are still in development, but that can be used to help us make the bridge from the high-throughput screening approaches to the in vivo systems.

DR. DRESLER: Dr. Guyton, I know you're on the phone and that's hard, especially if we have the technological delay across the ocean, but if you'll, I don't know, clear your throat and say hey, something, then I can call on you, okay?

DR. GUYTON: Yeah, I'd love to make a comment on this topic if I could.

DR. DRESLER: Please do.

DR. GUYTON: Sure. Well, I wanted to thank the questioner. I think this is a really, really important question, and I think some of the other speakers have given some really good insight into it. I would say, in terms of the evaluations that we're doing, we're really looking at how those data are consistent with or they may be supportive of other more traditional data, and we're also looking to where they may fill important gaps. So I think, you know, you can envision different roles for in vitro and other high-throughput testing data as to where we see a role.

I think it's very important to start to get some

experience. I mean, I think as Dr. Farland said, the report came from the National Academy 10 years ago, and I know they're working on another report, and we're very much looking forward to that to see where we can continue to go and make progress. But I think getting experience is really very helpful, and some of the insights we've gotten from some of our recent working groups that we've challenged to wrangle with these data, I think, will hopefully be very valuable to the community.

DR. DRESLER: Okay, this is, Dr. Wiecinski, a question for you. Your inclusion/exclusion criteria did not include relevance of route of exposure. Can you please discuss how the route of exposure may be considered in the weight-of-evidence approach?

DR. WIECINSKI: Of course. What I provided in the talk was really just a few short exclusion criteria that we use. Route of exposure, of course, is important and would be considered. I lost my train of thought. My apologies. Yeah, you always have to consider the route of exposure. It should be depending on your -- all that links back to what you define as your causal question.

So if your causal question is distinct to a specific route of exposure, then you should limit the studies that you're

evaluating based on that route of exposure. If it's more inclusive, then you might have some different problems where you would evaluate different routes of exposure, and it all depends on the question you're ultimately trying to answer in your weight of evidence.

DR. DRESLER: Okay. Okay, Dr. Rice, some of these questions are longer, okay? So let me look through it, and I'll try and pull out the real question to ask you, okay?

So you showed a PCA for engine exhaust data from 11 lung tox tests. The first PC explains 90% of variation and basically splits high/BOD/light gas scenarios from more typical scenarios. Isn't this expected? What do you really gain in insight from this analysis? Does this really help in the hazard assessment from mixtures? So the question is the first PC explains 90% of the variation and basically splits between high, bad, and light gas scenario from more typical scenarios. Isn't that expected? What do you really gain in insight from this analysis?

DR. RICE: I think the statistics in this particular case kind of showed what you would expect. So I think the interesting thing to me was that it could be done, you did see differences, and as a result, you could actually say, well,

this is a distinct group from this group. I think that's, you know, really what it showed with the statistical approach. I think the insight really is then to begin to develop, you know, further analyses as to what distinct components or what assays do they really light up in this particular PCA, you know, again. So that's the way I would address that.

DR. DRESLER: Okay. Anyone else want to address that?

(No response.)

DR. DRESLER: Okay. Dr. Guyton, this is for you. When you discuss evidence in humans, what type of studies are evaluated?

DR. GUYTON: Yeah. So thanks for that question. It's a really good one. The evaluation of evidence in humans is a systematic review, so it would include any study that would be identified. It really depends on the agent. It might include, for example, studies of workers. It wouldn't have to be really of the exposed population, and certainly that would be the case for the evaluation of many of the tobacco products. I hope that answers your question, but I'll be happy to clarify if not.

DR. DRESLER: If it didn't answer the person who asked the question, they get another card and they can ask it. So that

will take care of that one.

Dr. Farland, what factors may be considered to evaluate if endogenous levels of a chemical are relevant to exposure levels that result in an adverse effect? So what factors to consider to evaluate if endogenous levels of a chemical are relevant to exposure levels that result in an adverse event.

DR. FARLAND: So there have been a number of approaches to try to deal with this kind of a question. First of all, I think it's important for us to recognize that endogenous levels may very well be different in human and animals. And so this question of bringing the endogenous levels into an exposure or a total dose estimate when we do risk assessment depends on our ability to get some data for the species of interest. And so I think the kind of data would be what kind of normal blood levels one might see in a metabolome for some of these sources of normal exposure that would come through the diet or through the metabolic activity of the microbiome, for instance, metabolism itself maintaining particular levels. So those would be the sources of the chemical.

The second thing would be the regulation of those chemicals. And so are there -- do we understand something about the homeostatic processes that are in play that allow

these to be regulated so that when there becomes an exceedance, are there opportunities to bring that back into the normal range, and over what time period and what kinds of activities are we discussing?

And then I think, finally, the question here has to do with some of the issues, one of which I alluded to for formaldehyde, which is access of the endogenous chemical to a target versus the exogenous chemical from the standpoint of relative access, depending on where the formation of those chemicals are taking place.

DR. HOENG: If I may just add, I think also in light of the previous presentations, you know, the omics technologies were mentioned quite a few times. So we found it very valuable to actually -- particularly in rodent inhalation studies, to complement them to what we call a plus part.

So you basically tend to look at the respiratory organs as well as liver and perform gene expression as well as lipid profiling because you're actually enabled, then, to look at the molecular changes that are induced by aerosols and those you may see, even though histopathologically you may not necessarily have observed, you know, a change. So leveraging some of these omics technologies for low levels of carcinogens

or really significantly reduced products, it's a great strategy, I think.

DR. FARLAND: I think that's a really good point, and one of the things that we are faced with is that so many of our tox studies are done at high doses that we may very well demonstrate a hazard at those high doses, but we don't address this question of a low dose response or, in fact, an additive response between endogenous levels and an exogenous exposure. And so this becomes particularly important as we're beginning to think about risk. So the omics technologies allow us to take more of a bottom-up approach to this type of assessment as opposed to our typical top-down, which may very well have overwhelmed the issues related to endogenous levels when we look at either animal or human populations.

DR. DRESLER: And perhaps this follows on. So a little bit of a long one also. A primary consideration has to be duration for tobacco products. Most exposures might best be described as a repeated episode of acute exposures, often for a considerable portion of a lifetime. By definition, in vitro studies are acute or short term in duration. How can the mechanisms as a result of repeated assaults be examined via in vitro assays?

Go for it, Dr. Farland.

DR. FARLAND: So some of the examples that I showed used approaches where they looked at gene expression or the omics over a period of time, at 1 hour, 4 hours, 24 hours, and looked for whether or not there is a persistent result that comes about, and I think that will continue to be an opportunity for us. The presentation that Lynne gave actually addressed some of these issues with regard to how one begins to look at the question of a cumulative dose and whether or not there is recovery between individual doses or whether or not there's persistence of an effect that essentially resets the level, if you will, the new normal that I was describing, that eventually will produce a response with repeated exposures.

DR. HOENG: Right. And as was already indicated, I think the cell culture, really they're starting to really advance. So maybe at the air-liquid interface, we're not yet able to run months long of experiments following the exposure because you may get also contamination. But in the future, hopefully organ-on-a-chip platforms where you have an enclosed system, you could imagine, to expose your culture, maybe a lung culture, and keep it in the plate and then basically investigate the impact on liver metabolism by sort of setting

up your system where you do micro-sampling. So it's really like a miniaturized human on a plate.

But also we have done some of these experiments recently. You can also work just with BEAS-2B cells and perform sort of repeated treatments of cigarette smoke extracts, and we managed to do that for a period of 3 months. It's very labor intensive, and you need lots of lab technicians, but it is possible. So I think we're going to get in the in vitro world also to a place where you can run sort of 28-day studies, at least in vitro. In other settings, in the cosmetics industry, it has been done.

DR. DRESLER: So a question for you. So 28 days is different than lifetime. Would that be an accelerated process that you'd be looking at the organ-on-a-chip in the lab for 28 days?

DR. HOENG: Yeah, I think it would still be an acute toxicity kind of study, but you would have more sort of disease endpoints. You would not be mimicking necessarily an entire lifetime study like you would do in an aging mouse carcinogenicity study. So I think always the question is what is your hypothesis that you have, and what's the best experimental system available?

DR. DRESLER: Dr. Farland.

DR. GUYTON: Can I just comment here, if I may?

DR. DRESLER: Okay, please.

DR. GUYTON: Yeah. So I think this is a really good question. I did want to point out, though, with respect to some of the tobacco carcinogens and some of the tobacco evaluations as well as other carcinogens that IARC has evaluated, obviously, there are instances where you have a long-term exposure to a low dose, and that's leading to carcinogenicity. But it is possible to have a high acute exposure or sometimes not such a high exposure but that is acute that then leads to carcinogenicity.

And I think you can think about instances of, for example, those very sensitive windows during development and where in utero exposures don't require a long time-repeated exposure to really get to carcinogenesis. Certainly with some of the agents, we have evidence that this is really a few or maybe even some very discrete exposure of shrinking of your time window. And I think also we have some evidence from other types of exposures where this can be the case even in adults.

So I think there are some very, very different exposure scenarios to envision and to think about where toxicity data

from in vitro systems and other novel experimental systems can play a role and can sometimes be envisioning some of those other scenarios that are different from the chronic long-term bioassay data.

DR. DRESLER: Thank you.

Dr. Farland.

DR. FARLAND: Just to add. I think it's important to remember that in addition to the in vitro work that we would do, we can also do these omics technologies in intact animals with different exposure regimes and so on. And so the in vitro results may be hypothesis generating. They can then be looked at in more traditional animal studies through the omics technologies to look for similar sorts of things under a more standard exposure regime.

DR. HABER: So this is a very important question, and there has already been a number of very good comments from my fellow panelists. I just wanted to add a couple of things. One is with regard to the comments of Kate Guyton that, yes, this issue of not only focusing on the long-term effects, I think, is very important.

Sometimes I'm concerned, when we're focusing, people are looking at the time-weighted average, and as I noted, often

it's that peak exposure that's very important, that short-term exposure and particularly when we look at effects on children. I think that due to childhood being such a small percentage of the lifetime, that for chronic exposures I'm less concerned, although we do need to be careful about protecting children. But for acute exposures, we really don't, I think, consider sufficiently the differences in sensitivity between children and adults.

And then the utility of these longer-term in vitro assays, I think, is very useful for addressing this issue of what are the impacts of longer-term exposures?

One of the things that I found fascinating about some of the data that's come out with regard to prediction of longer-term effects is work from Rusty Thomas and colleagues, now at the EPA, where they were finding that effects during that first month of exposure and the gene expression changes there are often very predictive of the effects and the effect levels from chronic exposure. So, from that perspective, I find that reassuring.

And also just reminding us that we have the classic tools of risk assessment in toxicology that are also useful for addressing this issue and thinking about the toxicokinetics and

the toxicodynamics. So often I hear people talking about, well, the chemicals cleared quickly from the body, which is considering toxicokinetics, and the impact of short-term exposures are important, but we also need to be considering the toxicodynamics, so how quickly any damage is repaired and whether we can really consider each exposure incident as being a unique incident or whether there is a cumulative impact of all of those exposures.

DR. DRESLER: I'm going to take the moderator's prerogative because we have so many good questions and discussion. So because I didn't do a very long introduction of Dr. Ashley this morning, I saved us a lot of time. So if I can please have like 10 more minutes on this blinking light, please, because I have like at least 10 more questions to go, all right? And we'll still get to lunch before noon, okay?

Okay, so this next question, Dr. Guyton, is for you. Did IARC consider the contribution of endogenous chemical formation on a chemical, for example, the evaluation of formaldehyde or -- the other one I can't read, but let's just go with formaldehyde. So that's also a hint to everybody, please write it legibly. So, Dr. Guyton, formaldehyde, did IARC --

DR. GUYTON: Yeah, formaldehyde. Well, perhaps it's in

French and it's formaldéhyde, but in any case I'll be happy to answer it. The IARC evaluation of formaldehyde is based on the evidence from epidemiology studies of people who are exposed to formaldehyde. So this issue of the endogenous formation and so forth is very interesting, and I think it's an interesting scientific debate, but really epidemiology is kind of, you know, did A cause B? And in the case of formaldehyde, the answer to that question is yes, it's all -- the types of cancer that it does cause is another factor that does cleave to that. So --

DR. DRESLER: Okay, thank you.

DR. GUYTON: -- in short, the answer is no, we didn't really look at that.

DR. DRESLER: Okay. Dr. Haber, you provide equations with the assumption that a chemical is at a steady state. You also -- I think it says recent ambushed, and those two words usually don't go together for me in this question, so I'm sorry. You also referenced a recent published paper on intermittent exposures. Can you please discuss the relevance of intermittent exposure on the assumptions of steady state? So there's a recent paper that you spoke about. Can you please discuss the relevance of intermittent exposure on the

assumptions at steady state?

DR. HABER: So the equations I showed, based on the assumptions of steady state, are what is in the EPA's guidance for developing reference concentrations which are based on continuous lifetime exposure. One of the challenges is that, as noted here, you may not always be at steady state.

The intermittent exposure approach: A key purpose of the framework that I referenced is for when you have exposures that, of course, are intermittent and looking at whether one needs to also -- in addition to comparing the exposure level with an exposure limit that's most relevant to that exposure duration, whether one also needs to be comparing it with the longer term that reflects the intermittency.

So part of what we're looking at in the -- in order to say that one does not also need to -- so the automatic approach would be if you have a short-term exposure that's repeated many times, that you would be comparing that exposure to the exposure limit that's relevant for that duration. So for example, if you have -- now, this was developed initially in the context of hazardous waste sites, so you may have an exposure for 1 week that's repeated every 6 months for many years. And so in that case, you would compare that exposure

with an exposure limit that's relevant for 1 week, and then the question is do you also need to compare that with an exposure limit for -- there's some background noise.

DR. DRESLER: Yes, but I think you're doing okay.

DR. HABER: Yeah, for an exposure limit that's relevant to the chronic exposure. And so there we consider the aspects of kinetics and dynamics. So is the chemical cleared from the body between the intermittent exposures? And also from the dynamic perspective, has the repair occurred?

So, basically, as far as the steady state, you're looking at after -- we're looking primarily in this framework for what's happening between the exposures, assuming that the exposure limit that is applicable during the time of exposure is appropriate. So it is a fairly simplistic but fit-for-purpose framework to address that specific question.

DR. DRESLER: Okay, thank you.

Anyone else to address that? Dr. Guyton?

(No audible response.)

DR. DRESLER: Dr. Rice -- I'm sorry, Dr. Guyton, did I jump too quick?

DR. GUYTON: No, I'm fine.

DR. DRESLER: Okay, thank you.

Dr. Rice, when a mixture contains thousands of components, any or all of which can potentially interact, can a "similar" mixtures approach be applied? If so, please comment or describe a potential approach. So contains thousands of components, tobacco smoke contains thousands of components which can potentially interact, can a "similar" mixture approach be applied?

DR. RICE: I'm going to say I think it depends, and I'll give you a couple of --

(Laughter.)

DR. RICE: So I think the things that it depends on is how consistent are the various toxicity or other types of tests that you might be looking at. Do they give sort of similar signals, similar signatures across the mixture that would lead you to kind of suspect that they would be similar versus perhaps others that would be quite different?

So I think it really is just going to depend on, you know, the types of mixtures that you're dealing with and how consistent or inconsistent they are across the different groups, you know, without sort of going into sort of a number of different layers. I think that's sort of the short answer to the question.

DR. DRESLER: Okay.

DR. RICE: But I'd be happy to elaborate.

DR. DRESLER: Anybody else want to try that one?

(No response.)

DR. DRESLER: No, they're sitting back. Okay. Okay, what are the projections for future work on whole animal and human validation of high-throughput data predictions? So we were talking about organ-on-a-chip or different other cellular studies. So what are projections for future work on whole animal or human validation of high-throughput data predictions?

Dr. Hoeng.

DR. HOENG: It's certainly an interesting question, and we've given this a lot of thought, actually, in Philip Morris because -- I think the microphone is still okay -- because our risk assessment, at least to some extent, is based on systems toxicology based data, and these are very large datasets. So we've tried to work with the scientific communities on developing standards, best practices for data sharing, giving the data out to the community. In addition, we're running what we call sort of crowd verification. So we're trying to engage the scientific experts to actually help us process some of the data, because whether it's gene expression, proteomics,

metabolomics, all tell me that you use a validated instrument and you annotate your datasets. Well, you actually have to align the computational methodologies to actually evaluate these data. PCA plots are great, but they are really only the starting point.

And ultimately you would like to gain mechanistic insights and to look into mechanisms. You have to develop biological network models. A lot of the science is still emerging, so we're also engaging here experts to actually build some of these biological network models for lung disease in particular.

And so we believe, you know, use the power of the crowd to verify the data, verify some of the conclusions, and work with the academic community in particular, as well as some of these technology platform providers, to actually develop jointly best data-sharing practices. And there are more and more scientific journals also emerging, like *Nature* or *Big Data*, scientific data that actually facilitate a lot of that transparency.

DR. FARLAND: Yeah, I certainly agree with those comments. I think the idea that we can develop biomarkers with some of these gene sets that we're talking about that can be tested in animal transcriptomics or, for instance, in the case of inhalation, lung lavage of human cells that might have been

exposed, give us some handles on ways that we can actually get at whether or not we're seeing similar things in vitro in animal or human systems. And so, again, as the technologies for doing these studies improve, we'll be able to do even better work in whole animals and perhaps with human cells.

DR. DRESLER: One last question that I'll ask before I get another. This was for Dr. Wiecinski. You note expert judgment is critical in a weight-of-evidence evaluation. Judgment may be subjective. Can you discuss methods to account for subjectivity in this process?

DR. WIECINSKI: That is a very good question. Of course, expert judgment is subjective. It's based on a lot of inferences, various data, of course, and I think it's rather challenging to remove the subjectivity from expert judgment. But that's why it's critical, as you make your expert judgment, to clearly explain -- point out here's my conclusion based on X, Y, and Z, whatever inferences I might have, whatever whatever, and also underneath that having the alternate biologically plausible conclusions that maybe a different expert could reach with the same data and sort of explain why you eliminated that, you know, this didn't make sense to me because of this data or that, such that when the next expert

comes in and reads, they at least know where you started, and then hopefully the two of you or five of you can have a fruitful conversation about maybe who's right and who's not or, you know, what things are considered more.

DR. DRESLER: Okay. Lynne.

DR. HABER: An excellent question and excellent response. I just wanted to add that this is also an area where peer review is really important, that having people from different perspectives, the sum really is -- the total is much greater than the sum of the parts here. And also, as far as addressing the weight of evidence for our alternative hypotheses, this is one reason that documentation of what was considered in a peer review is also very important and not just saying we agreed or disagreed with the initial assessment that we're reviewing, but here are other alternatives that we considered, and here is why we excluded those, and we also considered these aspects, but we did or did not agree with pursuing them so that, again, the totality of the information is very transparent.

DR. HOENG: I think the other aspect I would like to add, that there is also now journals, like *Faculty of 1000*, that are very prominent and the fact that you share your article and the reviewers have to give the feedback, but it's openly shared so

you know who's giving the feedback and what are the comments, and then you actually openly reply to them so that peer review is happening in a transparent manner. So that's one aspect.

The other aspect is, indeed, for a lot of the science that we embark on, you need experts, and we've been thinking, ourselves, how do we engage experts in a manner so that they remain independent? Especially systems toxicology, it's a very multidisciplinary field, and you need toxicologists, you may need disease experts, medics, people that understand big data computational methods.

So we've been working with a third-party company that has been kind of enough for us to recruit independent panels of experts from particular geographic regions that take a look at our data and our results and our conclusions on an online platform, and thereby the experts debate among themselves, they have a panel of questions, they review our data, they provide feedback amongst each other, and you know, they're debating, and at the end we get a report from their discussion, and if there are points that are open, we can address them. But you do want to have the experts actually look at it and give them enough time to do so. I guess it's very similar to panels of risk assessment.

But having everything online with modern technology so that people can actually look at the data and have the time to do that when they like, whether it's a Sunday afternoon or a Monday morning, you have to -- you have the capabilities to provide people the opportunity to actually review the data.

DR. DRESLER: And those are public, those online panels?

DR. HOENG: So these online panels have not yet been public, but we're about to share all of that, yes.

DR. DRESLER: And so I have one more question. Dr. Haber briefly mentioned chemicals -- Dr. Haber briefly mentioned chemicals lacking scientific data or data-poor chemicals. How does this lack of information influence the ability to develop an AOP framework? Since there's so much that we don't know, how do you take when you don't know, and guess what, you've got to make a decision?

DR. HABER: So I think that addressing the -- particularly in the context of the issues related to this workshop, addressing the data-poor chemicals is one reason that I think that these mixtures approaches and the in vitro testing will be particularly useful. And that's where the AOP -- one area where the AOP framework has particular strength because you test -- there's the potential for testing a very wide variety

of targets using this to generate hypotheses and then to hone in on specific targets and issues.

So it's certainly a big issue, and we do need to be careful about these uncertainties and unknowns, but particularly where the sorts of questions that the Center for Tobacco Products is addressing, that that helps to define the issues where this could be useful.

DR. DRESLER: Dr. Farland.

DR. FARLAND: I guess I would just add that it's important to remember, and several of us have said it, that the AOP itself is agnostic to the chemical. And so it's not that these unknowns would be generating AOPs; it's that if we get limited data on something that hasn't been tested, we may find that there is more or less evidence that it fits into a particular AOP as opposed to actually generating an AOP. The general sense is that chemicals don't produce unique responses but actually fit into a series of responses in biology, and I think we're all getting more comfortable with that idea.

DR. DRESLER: Anyone else? Dr. Guyton?

DR. GUYTON: Yes, I think this is a really good question, and I think I appreciate the thoughtful answers already from the panel. I would also just raise that there are a number of

predictive models that could be employed that are very helpful, at least in prioritizing agents for testing, that look at things like chemical properties and similarities. And some of those have become quite sophisticated with the ability to bring on biological data once they become available to kind of refine the model and to improve the outcomes. So I think this is an area where I think it's very, very helpful. At IARC, we certainly have recently done some automated text mining and queries of databases, and this kind of thing to help us prioritize, from among a large group of chemicals, to try to figure out where are the data, where there are not data, and what's the relationship chemically between the things where we know things and the things where we don't and also with the strength of the evidence. And we already do have it for things, at least, we already know about, and how does that all relate.

So I see there's a lot of opportunity in this area moving to kind of a larger database-type thinking as to how we can integrate a lot of different types of evidence, including about chemical properties, to make predictions and to prioritize agents for evaluation and testing.

DR. DRESLER: Okay, thank you. And I actually had more

questions, so I'm going to talk with our toxicology colleagues and see if we can't fit them in future panels. But I did say we'd get to lunch before 12:00, and we're getting really close to that.

Thank you very much, the panel and the speakers, for a fantastic session. So thank you so much.

(Applause.)

DR. DRESLER: Lunch is upstairs around the corner in that cafe that's there. I haven't known them to be fast, so if you're going to go to lunch, do please go up there because we'll start at 1 o'clock to come back here, okay?

Thank you very much.

(Whereupon, at 11:54 a.m., a lunch recess was taken.)

A F T E R N O O N S E S S I O N

(1:04 p.m.)

DR. DRESLER: So apparently after that great first session this morning, several people were saying, yes, but if we could only address exposure. Well, guess what? So this next session is on Exposure.

So the next speaker is going to be Paolo Boffetta, who is from the Mount Sinai School of Medicine, and he will be speaking on Data on Exposure for Tobacco Products.

DR. BOFFETTA: Good afternoon. So I would like to thank Carolyn, Susan, and David Ashley for inviting me to this very, very interesting workshop.

So I will have an overview of the data that are available on tobacco, exposure to different tobacco products in the U.S. It will be a very high-level type of overview. I mean obviously I have time to go into more details during the discussion, and I will try to address some of the cross-cutting type of methodological issues that need to be taken into consideration when people use these data and try to make sense.

So, broadly speaking, I think one can categorize the available data into the three different groups, three different types. I mean, there's administrative data that have been

collected for purposes other than health or, you know, tobacco, strictly related to tobacco health issues, particularly sales, administrative data, etc.

Health surveys, which form the core of what is used for tobacco use type of data, and there are many such surveys in the U.S. and in many other countries. There are international surveys now. I mean, there are many different sources.

And then a third type of data that is often used also are derived from epidemiological data, particularly longitudinal prospective core studies that have the opportunity, obviously, to link exposure data to outcome to risk data, but also provide usually access to the type of information that may not be captured on many health surveys and typically have these longitudinal dimensions, this opportunity for repeated measurements, etc. And there are, again, several large-scale longitudinal studies in the U.S. and in other countries, and I will use some examples here.

So I would address the three types of -- three sources of data and try to address, you know, strengths and limitations of each.

So the administrative data: These are typical sales data. These are some examples of what one can make out of these data.

These are the sales data for the U.S., the five major groups of products that have been sort of measured systematically across, you know, the last whatever, 60 years, as you see here, with the total products at the top and then the breakdown with the cigarettes, which obviously represent the majority, although it's becoming less and less true now.

Cigars: And now there are different types of cigars available in the more recent data, but if you go back before 1970, typically there was just one category, and then the other tobacco products, including the smokeless tobacco, the chewing and the snuff and the other things.

So if we look at total sales -- in fact, it's possible to go even farther back, and this is an example of data for total tobacco expressed as -- the biggest ones were adjusted, the actual amount was the total amount, I mean the total mass of tobacco products that were sold, were registered according to the sales data. This is a different way to express the same type of information expressed per gram of tobacco, total tobacco products per person, adult, or whatever per day, and we have a sort of similar pattern of this strong decrease, which is not captured, which is stronger than what is captured by the total sales because of the number of people in the population

has increased.

So the fact that tobacco product sales have gone down reflects a much stronger decrease on the individual level. But the interesting thing is that it's possible, with some caveats, to go back quite some time. So, basically, now we have one set of data of tobacco sales.

This is another way to present the same information. Instead of just having the amount, we can have a share of the market in total sales in the different products, and we have a similar picture from what we saw before with, you know, this decrease in the proportion of cigarettes and increase, in particular, in the proportion of cigars that seems to have taken place in the last decades in the U.S., and also to some extent the increase in the smokeless tobacco product, which very much reflects the fact that cigarettes has gone down as a category.

There are other characteristics of tobacco products that can be also studied, can be if data are available. These are a couple of other aspects of -- which may be important for health. One is the proportion of filter cigarettes of the total cigarettes, and here data are available since 1950 roughly, and I just brought them, you know. So that's a trend

which is well known, that basically cigarettes started to become predominantly filter in the '60s and then, you know, become almost exclusively filter cigarettes in the last, you know, 20 years or so.

This is another example of the characteristics of cigarettes that has changed over time, the proportion of menthol cigarettes, which reflects -- this is a proportion, so it's not necessarily an absolute amount but reflects obviously the fact that menthol cigarettes are smoked preferentially by African Americans and reflects the fact that the proportion of smokers has declined less in this group of the population compared to other groups. So the proportion of menthol cigarettes is increasing because the proportion of smokers of menthol cigarettes is becoming larger over the total. So now menthol cigarettes represent about 30% of total cigarettes sold.

And a couple of other characteristics: This is the amount or the average level of nicotine in cigarettes sold in the U.S. It's well known nicotine has gone down by about a factor of 2.5 between the '50s and the early '80s, and then there's been basically no change or very little change after that. So the actual level is about 1 mg per cigarette since, you know, the

last 40 years or so.

And I think I have tar also, which are very similar, a very similar trend. As you know, this has been regulated, etc., so to some extent this is really -- there's not been a spontaneous trend that has been following regulatory actions. And again, for tar, the level has been in the order of 12, 13 mg per cigarette over the last 30 years or so.

So strengths of these types of data: There are census data over the entire population. I mean, we don't have sampling issues, we don't have selection bias, whether it's what happens in a country like the U.S. or other countries.

By and large, the data tend to be comparable in different time periods, although core issues in terms of, you know, underreporting, illegal sales, etc., may change over time, so it may create some issues. But by and large, the methodology that has been used to collect this data has been fairly consistent, and these are publicly available data typically, so it's easy to access.

The main disadvantages apart from this opportunity for bias, because these are very -- is sort of a passive recording of data, you know, without any time to check the validity in terms of, you know, other sources of tobacco products to the

population that are not captured, for example.

The other major disadvantage is that there are limited covariates that can be used. You know, we can look at some major geographic distributions like sales by state, for example, but obviously we cannot go into the detail of the individual, that type of information, because these are not individual data.

Next, the health surveys, the tobacco-related surveys or more general surveys of different factors of health that include also information relevant to tobacco use, tobacco exposure.

The typical surveys: One of the most commonly measured indicators is the proportion of smokers in a given population, the population which has been surveyed, which can be a representative sample of the nation, a given sector of the population, or whatever. This is an interesting graph which was put together in this program called International Smoking Statistics, which has been run by Peter Lee, and he's an associate in the UK now for about 20 years, I think, where they really tried to compare all data of several aspects of tobacco use, including the prevalence of smoking.

This is the prevalence of current smokers in many -- in

all the available surveys done in the U.S., and you see there are about -- for men and women, I mean, the triangles are men and the squares are women. Obviously, these are medical surveys with different definitions of cigarette smoking with different age groups, different subgroups of the population.

So this explains to you, somehow, the variability within a very short time period, but you see some sort of general trend, which obviously is most likely reflecting what is happening in the population.

The interesting thing in this type of graphic, you see the amount of data that's available out there, and I think there are about 200 different surveys that are summarized in this figure, and you see the early ones are from the '40s, and then, as we move into the '90s and 2000s, you have many, many such points, you know, data points in any given year, maybe usually about five or even more with those.

If we look at one of these particular type of data, these are the National Health Information Surveys. These are more comparable, so one can try or at least, you know, attempt to really throw a line across the different points. These were data that were part of the bigger picture but just, you know, selected because they were part of one particular survey that

has been repeated over time in the U.S.

So we see the same sort of trend, you know. And obviously, the U.S. is a country where are so many such data that, you know, one can really play and try to get into the most reliable one. In other countries, the number of data points are much fewer, and the issue of compatibility, etc., becomes more of an issue.

So from this we see that, you know, if we believe that these are representative samples of the population with all the caveats because the response rate is far from being 100%, I mean, there are sampling issues, etc., still, we can consider that, you know, in the U.S. male, there has been a decrease from about 50% prevalence of smoking to about to below 30%, and the females is going down from 35 to whatever, 25%. This is what has been shown.

If we move away from cigarettes, however, and try to look at similar data for other tobacco products or total tobacco, the data become more scanty and a little bit more problematic to interpret.

This is the same type of summary when we looked at the percentage of smokers of any tobacco product, taking into accounting also cigars and pipes, and you see that -- you know,

we see similar trends obviously, but the number of data points is much smaller than for cigarettes. And there are some clearer issues, for example, here. In terms of surveys done in the same period, you're getting fairly different -- fairly inconsistent estimates, which probably reflects the methodology, etc.

Obviously, we have no time to go into the detail, but the point is when we try to look at aspects other than cigarettes, you know, we start to get into a bit less, you know, sort of strong data.

And this is the same for total tobacco users, including smokeless tobacco use. You see that, you know, most of the data really belong to the last 20 years or so, and before that there was relatively little information. Again, you know, we see variability across different surveys for the same, which are supposed to do the same thing.

Interestingly, when people look at number of cigarettes smoked per day per person -- again, these are individual data on surveys that were asking, you know, individual people -- we have much more consistent data, and the trend that we saw in the sales, you know, appears to be -- obviously, it's confirmed, and we have much more detailed information. And

quite reassuring, the different surveys seem to measure the same thing to a large extent. So I think this is much more believable as a type of data and less problematic.

These surveys provide us information on a number of other factors that are important. This is another example. This is a different survey, the Behavioral Risk Factor Surveillance System, which has been going on also for many years, run by CDC. So it's sort of the -- probably many of you are familiar.

This is one of the many types of, you know, results that you can derive from the BRFSS, and this is a well-known pattern of proportion of smokers in different states of the country, where you can see very strongly the effect of regulatory action that has been stronger in some parts of the country and somehow weaker or even absent in other parts of the country, in particular the areas of -- some of the areas of tobacco production, I mean, like obviously Kentucky, West Virginia, Tennessee, etc. This has been going on -- I think these are the data for, yeah, 2014. But, you know, you have many other types of information that can be derived from BRFSS.

These are some of the other examples. We can start to look, for example, at age-specific rates instead of looking at the rate in the entire population, trying to see what happens

in young people, in middle-age people, in older people. This is an example just comparing two of the NHIS, 2005 and 2013, but you know, just an example.

And you see that both in men and in women -- these are men in 2005 and 2013, and these are women 2005 and 2013 -- there has been this decline which has been quite strong in the young and middle-age group, much less so in the older people. So apparently people, when they get to older age, if they're still smoking -- although only about, as you see here, 5 or 7 to 10% of the population still smoke at that age. There's been no major trend in decreasing that prevalence in the last 10 years.

This is an example of a slightly more sophisticated way to look at the same data, again from the NHIS. It's a nice paper by Holford, some of the people involved in the lung cancer -- in several lung cancer projects where they looked at the prevalence of smoking broken down by calendar year and birth cohort.

So each line is a birth cohort to those who were born around 1890, those who were born around 1900, 1910, 1920, and then how the tobacco experience -- these are men, yeah, these are the men -- how the tobacco experience evolved throughout life of these people and when and where, time when they were 20

if we follow the first line, etc. And we see this, you know, happening of the tobacco epidemic somehow.

And I like this type of, you know, trying at least graphically on a two-dimensional type of graph to show the pattern, you know, and we see that, for example, the cohort of people born around 1920 are the ones with each of the highest prevalence of smoking, around 70% were about 20, 25. And then, you know, a subsequent cohort had a much lower rate, although we see that in the very -- in the more recent cohort like, you know, the one born in the '80s or the '90s, there seems to be a higher prevalence compared to those born just before that. So we can look, you know, at the evolution of these patterns in the population.

The same for women: This is based on age and birth and just the probability of initiation at any age for the different birth cohorts. So the birth cohort was born around the '40s, this red line, in the one which had the highest rate of initiation, in particular when these women were in their teens, of the late teens, you know, 17, 18, or whatever. And these are the very early cohort, the people, the women who were born at the turn of the 19th century, and there was basically nobody who smoked in that cohort, and then we see again, you know,

this happening. It's just a different way to present the same data.

This is one of the best known and best studied characteristics of the smoking rates in this country. It's by ethnicity, race/ethnicity. This is men, women, and the entire population, all ages combined. And we have this well-known higher rate in American Indians and the native Alaskan and then, in general, this higher rate in African Americans compared to whites, at least in men, less so women. Whites, Asian, and to some extent Latinos have a higher -- have a lower, sorry, prevalence of smoking, a higher prevalence of never smokers. And this is particularly true for Asian women, but also to some extent Latino women, Latinas.

Another aspect that can be easily studied -- and again, many uses at NHIS, but there are other data from other surveys -- is the pattern bifurcation, and we have this trained by which, you know, people with the highest education tend to have the lowest smoking rate, although there are some variabilities, particularly in women, where women with very low education seem to have a lower smoking rate or so. It can be confounded to smoking.

Poverty level is still another factor that can be

considered in these surveys. And again, you know, people below poverty level tend to have higher smoking rates, but this is well known, both now -- I mean the more recent data, 19 -- sorry, 2013 and also largely 5 years ago, and the decrease in smoking prevalence has been mainly in people above poverty level.

This is still another survey, the national study for drug user, and this is one of the examples where we have data on smokeless tobacco, and this is the prevalence of smoking tobacco used in different periods and in different calendar periods in men and women. But again, these are just examples.

And finally, proportion of smokers who quit, I mean, but I don't think we need to spend much time on this.

Just before closing this and going to the epi studies in the last few minutes, one of the studies that's been used a lot also to study tobacco use is NHANES, this survey that is repeated every 2 years on about 15,000 to 18,000 people, again, supposedly a representative sample of the population in which not only data are collected on different habits and to the smoking, but also biological samples are collected and analyzed for a number of biomarkers.

And this is a nice analysis done by people at Stanford a

few years ago, in which they look at all the different biomarkers that were measured in NHANES. There were 188 of them, including our friend cotinine here, so the biomarker of recent tobacco use. And the interesting aspect of this analysis is that they look at all the correlations between the different -- these different biomarkers in the people. I think this is the NHANES. I think it is 2002-2003 data, if I remember. Yeah, it should be that one, anyway, in one of these cross-sectional analyses. So the point is that, you know, these markers don't travel alone; they travel together. There are strong correlations either positive -- and these are the blue squares. You know, each of these dots is a correlation of one biomarker with another one, or negatively correlated, the red bar.

And cotinine, in particular, is this line here. So cotinine tends to be correlated with many other factors, to some extent because many other toxic agents are brought -- people are exposed to toxic agents through tobacco, so that's not surprising, but also because smokers tend to have different habits than the rest of the population. So we have to keep this in mind when we look at biomarker data, that there can be confounding or there can be correlations with other factors.

Okay, another issue to consider is precision. Some of these surveys are large, but not so large. And, for example, these are the same data of smoking prevalence by race that I showed before, but this time I added the confidence intervals into the estimate of the prevalence. And you see that for some of the groups, in particular the American Indians, you know, the ones with the highest rate, the confidence interval is quite large because of the number of smokers on which these data -- on whom these data are based is not so large. I mean, they talk about a few dozen people probably in the entire survey, although for some of the other groups, the data are more robust.

So strengths of health surveys: the representativeness. There is a big effort to try to get, you know, a good sample that really allows inference to the entire population that tend to have detailed data on tobacco. We saw many examples of some of these additional data that can be used and, to some extent, data of covariates and biomarkers. So these vary differently from one survey to the other.

There may be data on self-reports, so obviously the validity of self-reports, there is big literature on that. There is heterogeneity, and we saw some of the examples, you

know, where you get these very variable results. Some of them are not very large, and then they are cross-sectional. So you measure the population at that point in time, but you don't know what that is, you know, before and after. There are often no repeat measurements and no linkage with occurrence of disease.

So finally the epi studies, and I just use one example of the American Cancer Society Cancer Prevention Study II because this study I like a lot. I've been working a lot with this data during my career.

This is an example of an early analysis published by Steve Stellman, now 30 years ago, on the proportion within this big study of more than one million volunteers. The proportion of tar level in the cigarette smoke by -- the men's cigarette smoke by the people according to different educational categories. And this was one of the early examples, you know, of how much education sort of correlates with a possible toxic effect of tobacco.

And this is another example of one of the early analyses we did in this data, was the prevalence of smoking in different occupational groups. So in these epi studies, beyond looking at the risk aspect, you know, the longitudinal aspect, it's

possible to look at other dimensions of the tobacco use issue. For example, in this case we look at, you know, which were -- how tobacco was distributed in different occupational groups.

So the epi studies have the advantage of having a large number of covariates, usually have biological samples of data, so it's possible to look at biomarkers and especially to have prospective designs. So many of them had repeated measurements. You can see how people changed behavior and how this would affect -- you know, this is a richer sort of, you know, type of information. Again, these are datasets as reported.

There is the issue of representativeness. These are not random samples of the population; these are samples of one key estimate. People tend to be healthier, etc., etc. So there is a whole issue of how to generalize to the entire population.

So my conclusions: Obviously, we have a lot of data on tobacco exposure, particularly in this country and in many other countries in the world. Sources of information are variable, and it's very important to assess their quality and the possibility of bias, precision, missingness of data, selection bias of the population, even if it's presented as a representative sample, and measurement error. And obviously we

can elaborate in any of these issues during the discussion.

Thank you very much.

(Applause.)

DR. DRESLER: Thank you.

Our next presenter will be online, and so we'll -- I'm looking for a nod. And there, I'm not going to touch the computer, but it sounds like -- Dr. Klepeis, are you online?

DR. KLEPEIS: I'm here, yes.

DR. DRESLER: Okay, so Dr. Klepeis is from San Diego State University, and he'll be speaking on Methods of Assessing Individual and Population Exposure to Secondhand Smoke.

And so if you'll just say next slide, we'll go ahead and change it here. Please let us know if you're getting a delay, but since we're on the same continent, even though we're pretty far apart between here to California, hopefully it will still work smoothly. So, Dr. Klepeis, you're on.

DR. KLEPEIS: Okay, thank you very much. I don't see any slides yet. I'm not sure if there's any trouble getting them online yet.

DR. DRESLER: Okay, so I'm getting one finger that says 1 minute. So it is a good finger, and it says hold on 1 minute.

(Laughter.)

DR. KLEPEIS: Okay. Well, I could introduce myself a little bit.

DR. DRESLER: Okay.

DR. KLEPEIS: My talk will be a little bit of a change of pace, I think, coming more from an engineering perspective with secondhand smoke and exposure for users and nonusers of tobacco products. So I'll be focusing largely on the methods of models that will plug into total risk assessment.

DR. DRESLER: So they're working on the technology in the back. So, Neil, let me know if it's popping up. I'm seeing it on my screen now. Do you see it on yours?

DR. KLEPEIS: I see a blue screen.

DR. DRESLER: It might be the blue screen of death, or is the blue screen with your slides on it?

DR. KLEPEIS: Windows blue screen it looks like. Here we go, it's there.

DR. DRESLER: You got it? Great, all right. Thank you. Go ahead.

DR. KLEPEIS: Thank you so much. Okay, yes, I'll be talking today about various methods for assessing individual and population exposures. So I'll start by looking at the data

and the methods for measuring exposure in the field and moving that into some models and larger -- in a population-based model.

So the next slide. Do I have to press something or --

DEREK: Dr. Klepeis, this is Derek. I'm part of the AV support here. I'd just like to let you know that we are advancing the slides in the room, but if you're watching online, there will be about a 10-second delay. So if you just want to go ahead with your presentation, that would be great.

DR. KLEPEIS: Okay. So the second slide here is just giving a little bit of background for me. I come from an engineering and physical science background, so basically my career and my research involves measuring exposures, levels of pollutants in the field, looking at activity patterns and creating models.

And the next slide after that is the outline of my talk. So I'll start off a little bit with the building blocks of what exposure risk and dose are, just to frame it and scope it out for this particular talk. I'll then move on into some of the established methods that folks have used over the years to look at personal and the area measurements that lead into exposure for individuals and populations. And there are a lot of

exciting things coming down the pike for emerging methods to assess exposure, so I'll just touch on those briefly.

The next slide here is an equation. It says risk is equal to exposure times hazard. I just want to frame this out. I know this is sort of a very simple beginning concept, but I want to be clear on what my talk is going to be focusing on.

So risk is a probability that some kind of exposure to a hazard will lead to something bad happening. As exposure goes up, then risk goes up. So we're very interested in what exposure is, what the magnitudes of exposure are.

The next slide is the equation exposure is concentration times time. So this is a fundamental building block that exposure scientists use. The concept that we hinge all our exposure assessments on is the idea that an agent is coming into contact with a target, and that target is being exposed, and exposure goes up as the concentration goes up and as the time being exposed goes up. So we're interested in both of those quantities when we do exposure assessment, the level of exposure that's occurring and how long that exposure is taking.

Finally the dose slide, the next slide. I won't be talking about dose today, but dose can be -- it's sometimes tacked onto these exposure models, so the amount of the mass of

the agent that is crossing a certain boundary, so how much is depositing on the lung, for example, how much chemical is crossing the boundary to the blood. All of these things can be, you know, a component of a larger exposure model, per se, but it's not technically an exposure. So I'll be really focusing just on the exposure part of that today. I'm not going into dose. That's a whole other can of worms really.

On the next slide, I'm just emphasizing a little bit more how, you know, we're looking at smokers and that smoke mixes in the air and then is inhaled into the lung. So we're looking at really the amount that's at the breathing zone of people in the room or the space around the smoker. That could be a smoker, the smoker themselves, or nonusers in the vicinity.

On the next slide here, I just wanted to summarize a little bit of the relevance of, you know, how exposure assessment fits into the tobacco -- the evaluation of tobacco products and regulation of them. Every time we use cigarettes, cigars, pipes, vaping, we're emitting pollutants into the air. Those pollutants are mixing, and both users and nonusers can be exposed to them. We know that there are a lot of hazards in tobacco product emissions already. I won't be talking too much here about the specific components but more about the bulk

exposure, but all of these methods can be applied to any specific compound or species that's in tobacco smoke. We tend to look at particulate matter, nicotine, carbon monoxide, things that are somewhat easier to measure, but you can apply these methods across the board.

The next slide summarizes really the components, the elements of assessing exposure. First, you need some empirical measurements really to get some ground truth on what we're talking about here. So we need some pollutant measurement devices. That's the first component, the concentration component of exposure.

Secondly, we need to have some idea of the activity, the human activity pattern, you know, what people are doing, what the source patterns are, what the receptor patterns are, and where people are, their physical activity, what location they are in.

And then we can start looking at individual exposures of initial building blocks, personal exposure. If we look at what's happening in people's breathing zones, we can look at area sampling and use that as an indicator of what people are breathing in. These move into modeling, engineering models of spaces of buildings, and we use questionnaires on what people

are doing to kind of have an idea of what the source of activity and the receptor activity are.

So the culmination of all of this, I think, for our present topic, this assessment is getting an idea of the population exposure, how are people out there being exposed and what's the probability of distribution of that exposure. So we start using stochastic models and surveys of activity patterns, plugging all of these empirical and individual exposure models into this larger model. And that's what this whole process will kind of be leading up to, but at the end I'll give some examples of that.

But in the next slide I've integrated air sampling. I'll just start here and talk a little bit about these methods and then ramp up to the modeling towards the end.

So basically, you know, we use these filter and pump devices to collect particles of nicotine on filters, and then we analyze those in the lab, and we weigh them before and after. You can use a variety of different absorbent tubes and canister technology to trap air and the pollutants on a media. And then we analyze that in the lab to find out what the concentration was in the atmosphere during measurement.

The next slide is a smattering of different direct-read or

what we call real-time instruments for measuring exposure. These here are focused on particles. And you have the Sidepak and a lot of people may be familiar with the Dylos, which is a cheap particle counter which is gaining a lot of use in different studies. Things like the aethalometer or particle counters. The EcoChem instruments have been used quite a bit. We have direct-read instruments for all kinds of gases as well.

And interestingly, there's a nicotine real-time monitor that's starting to come online here, and I've been in contact with the developers. If that's reliable, it would be quite a useful way to pinpoint exposures from tobacco. Particles are not specific to tobacco. They can get readings from cooking and dust and all kinds of other things in the environment.

In the next slide I'm giving you just some examples here of what studies we've done in the past. If you go back 20, 25 years, I've really studied almost every single kind of environment, my colleagues and I have at Stanford, and Jim Repace and other folks all over the country and all over the world have started to really hammer on all of these different micro-environments to get a really good idea of what kinds of exposure levels can occur in all of these environments. So I'd like to highlight just a few of those.

Casinos are some of the more recent ones we've looked at, and we put the Sidepak instruments -- you can see the yellow circles where these -- a security guard and pit boss, they're wearing Sidepak instrumentation with tubes going up to their breathing zone. And I protected the identity of the innocent here. And so they walk around during their shift and get an exposure profile.

So another way we characterize exposures more indirectly in casinos is by using area sampling. So we'll put a monitor outside and measure for 24 hours. We were able to put these monitors in a cashier's cage as well. We were able to leave those in there for a whole day, and then we could look at the distribution of exposures of concentrations in the space, and we can infer different exposures from that and how much exposure is due to cigarette smoking versus maybe ambient pollution.

The next slide shows the profile of a person spending time in a casino as well as outdoors. So this is kind of an individual's sort of personal exposure profile in which we replicate the number of times it can provide important input into the empirical characterization of this micro-environment but then could be fed into a larger population exposure model

down the road.

So this is kind of -- it's sort of a time series experiment really. It lets us pinpoint what -- how drastic smoke is contributing to this person's part of exposure in this case. The outdoors you can see in the beginning of the time series, and we have a very low level. And as you move indoors, we get into the 100 to 200 $\mu g/m^3$ level.

And the EPA has a 24-hour particle standard of 35 $\mu g/m^3$, and this was really used as sort of a rough guideline for us to have some sense of how large these particles are getting when they communicate to different folks.

So we can just see that as you go inside, you're getting above that benchmark EPA limit, that you get to above 35 $\mu g/m^3$. If someone were exposed to this for long periods of time, then their 24-hour average could conceivably exceed that EPA limit as well.

So, on the next slide, I'm just showing the before and after levels, and this really gets to the point of where do all of these particles come from in the casino? So the panel above is when there is smoking allowed in the casino. The panel below is pretty much the same kind of profile and the same kind of activity pattern when smoking was banned in the casino, 100%

banned.

So we see the amount of exposures in the middle basically go to zero. So we can conclude from that that all the particles were very, very likely due to just tobacco smoking.

If you see these levels on either side in the lower panel, these are outdoor levels. So all smokers inside went outside. So when we went outside to sample this supposedly clean outdoor air, we actually got a lot of smoke there, and as you can see, those are fairly substantial levels that you get standing by a smoker outside. So that kind of an exposure could be incorporated into a larger population model as well, by taking into account how much people spend time outdoors in proximity to smokers.

Okay, in the next slide, I'm going to touch briefly on sort of a hot topic in secondhand smoke, which is multiunit housing. This is one study we did in a large high-rise in San Jose, California, and we put a monitor in a condo next to a unit where a smoker was active, and we were able to look at the activity pattern of that smoker. We witnessed when they came home and went into their apartment, and we were able to smell smoke with our noses, and we knew generally when the smoking occurred, and we were able to measure the level.

And in this next slide it says smoker living next door, daily pattern of levels approach that of a smoky casino. So we were able to look at it with a control and monitor another condo in a whole different part of the building. We were able to see how the levels increased in this room adjacent to the smoker, and those levels did get quite high, and this was sort of an extreme case for exposure to levels in multiunit housing.

So this kind of exposure, I think, is also pretty significant across -- you know, in terms of the number of people experiencing this and the levels can be high. So this could be something we would want to include in a larger population model as well.

In the next slide I'm starting to look at outdoors. More explicitly, we looked at the casino case, and we saw some evidence of smoking outside leading to significant exposure. We did a focus study on this topic 2 years ago, and we had people smoking in cafes, and we measured different distances away from them to see exactly what the affected proximity was outdoors.

In the next slide we show the effect of distance where a single smoker smoked outside. So we were able to see that these little spikes actually got over 100 or 1500 $\mu g/m^3$, and

the average over the cigarette was in the 200 to 300 range. So these are pretty high if you're near smokers.

As you get farther away, the levels go down. But if you're near a smoker, you can get exposed to pretty high levels that are somewhat comparable to or greater than what you might get indoors to a single cigarette. Indoors, those levels can mix and become dilute, and you don't necessarily get these large spikes. If you're downwind from a smoker outside, you're more likely to get these large spikes, and if you're directly downwind, then you might inhale some very concentrated plumes of secondhand smoke.

The next slide is looking at automobiles. We did a pretty involved study of carbon monoxide and particles in cars. We drove around with the windows up and down and at different speeds and the air conditioning on and off and all kinds of configurations to see what the levels of smoke and the ventilation levels could be in automobiles.

So this next slide shows the particle concentrations that we measured in the car at all of these different configurations, and you can see the EPA 24-hour hazard level also on this, just to get some scale to what levels we're talking about, which are very, very large.

So here in the car, smoking with the windows closed, you're getting up to 3-, 4-, 5-, or 6,000 µg/m^3, which is a very, very large level. So if you're a smoker exposed to that, or a nonsmoker, you're getting large amounts of exposure due to that secondhand smoke and that mixing of the emissions coming out of the cigarette. As you open windows or use HVAC or AC, I should say, the levels do go down, but you're still -- the peaks still do go above the EPA limit on the chart. So there's still significant exposure.

Okay, so that's some examples of the empirical investigation of exposure to tobacco smoke, cigarette, cigar emissions mostly.

The next slide is showing a mathematical model of the car. So we're able to describe the time periods and the average concentration of particulate matter in these cars, or carbon monoxide in these cars, pretty well by knowing the configurations, by knowing the ventilation rate roughly, and the emission rate of the cigarette and the cigar, which we characterized in other studies.

So we were able to understand or to describe the levels in these different micro-environments pretty well with an engineering model, so we could then use this kind of model

inside of a larger population model.

And in the next slide, here's some data for smoking a cigar -- I'm sorry, a cigarette in a bedroom of a house.

If you go to the slide after that, we were able to fit a model to data of that kind using carbon monoxide. In the other case it was particles. But we were able to fit a two-compartment model here to the data for a house. And here, the parameters are airflow rate in between the compartments, the volume of the room, the emission rate, etc. But again, we can describe the profile of exposure of room concentrations pretty well, as long as we know the ballpark values of the parameters.

The next slide.

We've done quite a bit of multi-zone modeling as well in residences where we can look at any number of rooms we want. As long as know the rough airflow, the air exchange rate for these rooms, we can predict with decent precision or decent accuracy what the levels are in the different rooms.

On the next slide is an example of the output of a simulation model of this kind, where on the top is one, two, three, four, five strips showing the real-time concentration in each room of the house. Below are the activity patterns. So

this really highlights the components of an exposure model, a micro-environmental exposure model where we have the concentration and the activity patterns together.

And then we can compute the smoker's own exposure. So if you look down just below the middle part of this figure that says Smoker Exp., 61 µg/m^3, this actually shows what the smoker would be exposed to due to their own smoking. And then below that is the nonsmoker's exposure. Taking into account what rooms they entered during the day, we are able to compute their profile for exposure.

So that gives an example of the kind of micro-environmental modeling you can use to build up a larger exposure model, as long as we have some idea of what concentrations are occurring and what the activity patterns are.

So I'm not sure if I'm running out of time here. It's not too much longer, so I'd like to touch on how we put all of this together to assess population exposure as input into a total risk assessment model. So that next slide just shows a little graphic of population exposure.

The slide after that is a little flow diagram for exposure, for what an exposure model looks like, how you build

it up. So at the top here, we have four basic components that can be combined for input into a population stochastic exposure model. We can have distribution, probability distributions of empirical data. It could be daily data, weekly data, hourly data. We can decide on what time scale we're interested in. If you went for lifetime exposure, we can decide on what spots we want to treat.

And as I've presented here in this talk, we can make sure of micro-environmental models for specific environments. We can choose to do that for environments that can be well characterized by a model, perhaps automobiles, homes. Things that are less well characterized, it's harder to get those parameters. We can use samples from empirical data as input into the modeling, the micro-environmental model.

We want to make use of building data, you know, how large these homes are. For example, how large the rooms are, what the air exchange rates are, the deposition rates, all of those kinds of things need to be input as well.

And then distributions of human activity patterns. There are a number of studies out there. One I was involved with a number of years back is the National Human Exposure -- National Human Activity Pattern Survey that was focused on exposure

factors or exposure micro-environments that are activities that would lead to exposure. There are a lot of other activity pattern surveys that are online, the American Time Use Survey and the others, that could be input into the modeling as well.

So we input all of these elements into the exposure model, and then we get a distribution of exposure for a given cohort or a given population, and then we can take that and plug that into some kind of a dose model and ultimately into a risk model.

So in the next slide here, I just want to focus on the use of these sub-models. So it's a very powerful way to do a sensitivity or an exploratory analysis of exposure to figure out what the most influential variables are, because we have many, many different models that we can use.

We have a lot of exposure factors. The EPA has handbooks available where we can get some sense of what these input parameters should be. We have a lot of activity pattern data out there. So this is, I think, a very powerful and useful technique to build up an exposure model and a risk model by using these mathematical sub-models that are based on empirical data.

As an example, the next slide shows some empirical time

activity data from the National Human Activity Pattern Survey that the EPA did around 1994. So on the left I just sampled about 25 individuals, and this shows, from midnight to midnight, what people are doing. It was a recall diary. We called people up and asked them, what did you do yesterday at midnight? What did you do after that? We had thousands of people that responded to that, so we were able to get distributions of activities, time of day, how much time people spent in different locations if they were with a smoker. So these kind of data are very conducive to building up an exposure model.

On the right is just an aggregate summary of the percentage of people in each of these locations by time of day. People, of course, spend a lot of time indoors sleeping, and then they leave to go to work for another day, then they go back home.

The last slide is an example of an exposure model, a stochastic exposure model, the SHEDS-PM model published by Burke et al. in 2001, from EPA. This is a Stochastic Human Exposure and Dose Simulation model for particulate matter, and they did a lot of what I've talked about here; they used a hybrid design, and they used models of input for the home.

They sampled some distributions for different micro-environments. They treated a lot of the common locations that were in the National Human Activity Pattern Survey, whether you were at home, in an office, in a school, outdoors, in bar, a restaurant. They built up this pretty sophisticated stochastic exposure model for Philadelphia.

So you can see the output on the right here of the frequency distribution of exposure for -- they broke it down into different subpopulations for some variables for indoor residential exposure, for exposure when there's no ETS, or secondhand smoke, or when there was. So you can see the general impact of smoking in the home relative to other particle sources.

So I think an approach like this could be very useful as input into a risk model, and we could do a lot more work, I think, now than they did in 2001 to pinpoint different types of exposure. Different locations, you know, casinos, cars, we can treat much better than we could in the past. So I think this would be a very worthwhile approach.

The next slide shows some of the technologies that are coming online, the whole idea of the Internet of Things and mobile devices. We have lots of sensors coming online and

things you can buy now for one or two hundred dollars that have a complete array of air quality sensors built in and then it uploads it to the cloud automatically.

So EPA and others are very interested in getting a lot of these sensors out and testing them and evaluating them, using them to assess exposure in the world. And these, of course, can be input into an exposure model, a population model, and to verify different micro-environmental models as well. So that's a pretty exciting development.

Lastly, there are a lot of other future directions in modeling and the whole, you know, push now and the convergence of augmented and virtual realities. All of these sensor packages that are coming online have really been used together to assess exposure and to gather data as well.

It's always hard to get time activity data and couple that with the concentration data. But with some of these new platforms coming online, we can get both at the same time, and we can involve people in sort of simulated environments where they can go through their day, and we can couple that with real sensors in the real world so we can really get a very good handle on what people are being exposed to and what the context for that exposure is.

So that's very exciting, and that's within my current push for research, is developing some agent-based approaches and some augmented reality approaches.

So that's it. I guess we'll have -- we can answer some questions at the panel later today. Thank you.

DR. DRESLER: So thank you.

Okay, our next speaker is going to be Dr. Ryan Potts from the Reynolds American Incorporated Services Company, speaking on the Assessment of Exposure to Tobacco Product Constituents.

DR. POTTS: Good afternoon, everyone. My name is Ryan Potts, and I work in the Scientific and Regulatory Affairs Group at RAI Services Company.

First of all, I'd like to thank CTP for organizing and hosting this workshop and for allowing me to speak today. Continuing on from the two prior speakers, I'm going to talk about assessment of exposure to tobacco product constituents.

First of all, here's my disclosure proudly starting the presentation.

So as we consider risk assessment of tobacco products, it should be clear, risk assessment is a tool that can be used as a component of regulatory decision making. It needs to be viewed in the context of other available data, whether that be

nonclinical data, clinical data, epidemiology, etc. And where it is conducted, it needs to be conducted with consideration for the regulatory pathways that are available for tobacco products.

And so what are these regulatory pathways that may invoke risk assessment of tobacco products? First of all, we have a substantial equivalence pathway whereupon a new product is compared to a predicate product, and the new product, in order to be cleared via this pathway, needs to have either the same characteristics as a predicate product, or if it has different characteristics, then it should not raise different questions of public health.

Secondly, we have the premarket tobacco product application pathway, which is suitable for new products that do not have a predicate product to compare to.

And finally, we have the modified risk tobacco product application, whereby a petitioner can seek FDA to authorize reduced exposure or reduced risk labeling and advertising for a product.

I shan't dwell on this slide since we've seen similar versions already this morning.

A simple definition of exposure assessment is to assess

how much of a chemical and by what routes are individuals exposed. For tobacco products, exposure can be affected principally by the chemical composition of the product or the product's emission, such as cigarette smoke, and secondly, by the degree and nature of the user's exposure.

As we've already touched on today, cigarette mainstream smoke is -- delivers a complex aerosol to the smoker. Several thousand constituents have been identified either in the vapor phase and/or particulate phases. The bulk of those constituents have been found in the particulate phase. Importantly, a composition of mainstream smoke varies with the cigarette design features and human smoking behavior.

Similarly, for smokeless tobacco, there are several thousand constituents that have been identified to date, and the composition, too, also varies with the product design features and the interactions of the user with the product.

Regarding the degree and nature of exposure, there is a wide variation in tobacco use behaviors, both within the same individual and across individuals, which I'll discuss more shortly.

As an initial crude estimate, tobacco product usage can be estimated simply by looking at number of snus pouches that are

consumed per day, for example, or the number of cigarettes smoked per day. Various tools are available to estimate tobacco product usage. However, these assessments do not allow for the impact of tobacco product use behavior, nor does it assess the constituent intake or uptake into the human body.

So what other approaches have been used to assess exposure to tobacco product constituents? First of all, we have product content analysis. So just for smokeless tobacco products, we have lots of data that's been collected over many years where machine-generated smoke yields have been presented, and these smoke yields have been assessed using machine regimes that attempt to mimic human smoking behavior patterns. We also have mouth-level exposure or intake analyses that have been conducted for cigarettes and smokeless products. And finally, we have biomarkers of exposure or biomonitoring of body fluids to measure uptake of individual constituents.

So, first of all, I want to talk about each of those in slightly more detail. So turning to machine-generated smoke yields, cigarette smoke yield data and more recently electronic cigarette aerosol data has been generated using multiple machine regimes over the years, and there are many publications that summarize that data. No regime proposed to date

accurately predicts constituent yields under actual human smoking conditions, and consequently, the determination of toxicant yields from cigarette smoke carries with it inherent limitations, for example, the concept of fixed machine conditions versus the variability in human smoking behavior, and consequently toxicant yields from machine smoking of cigarettes are not considered to be an index of a smoker's risk from smoking cigarettes.

So how can machine-generated smoke yields be used? This question was considered by an ISO working group and subsequently a World Health Organization study group focused on tobacco product regulation, and they concluded that smoke machine-based toxicant yields are useful to characterize emissions for design and regulatory purposes, and they may be used as inputs for product hazard assessment.

Generally speaking, smoke yields generated under Health Canada Intense puffing regime are likely represented at the upper boundary of potential human exposure to constituents. And finally, machine-generated smoke yields are particularly useful when conducting a head-to-head comparison of two similar cigarettes, particularly when product manufacturing and analytical chemistry determinations are temporally matched to

minimize inherent variability. Such a head-to-head assessment is often conducted during substantial equivalence assessments.

Moving on to human data, I'm going to talk about two approaches that have been used to estimate toxicant exposure among tobacco product users, the first being mouth-level exposure and secondly biomarkers of exposure.

So I'll turn to mouth-level exposure. This technique has been used to assess exposure to constituents in both cigarette smokers and smokeless tobacco users. It's a noninvasive methodology, so it does not interfere with normal product use behavior. It measures exposure in actual users under actual use conditions. However, it does not take into account inhalation or uptake of a constituent into the body. Having said that, measures have been correlated with biomarkers of exposure in tobacco product users.

Focusing first on mouth-level exposures to use of cigarettes, mouth-level exposure to various constituents from cigarettes is conducted by assessing the spent filters of a smoked cigarette. It provides a maximum potential exposure for a broad range of constituents in that the mouth-level exposure estimates do not factor in any exhaled smoke or mouth spill, for example, when a puff is being taken. Several constituents

have been measured and reported in the scientific literature, so just TSNAs and nitrosamines, nicotine, tar, etc. And as I'll show over the next few slides, the reported values show large intra- and inter-individual variability.

So this first dataset compares smokers' average daily tar mouth-level exposure with machine-generated tar yield values. The cigarettes were smoked on smoking machines using a variety of puffing regimes.

Starting over here on the far left, I'll just call out two of the regimes: the 35/60/2 puffing regime, so that will be a 35 cc puff volume taken every 60 seconds for a 2-second duration with 0% vent locking is the same as the FTC or ISO puffing regimen, and that's this one, this guy here; and then over on the far right, the 55/30/2 with 100% vent blocking is the same as the Canadian Intense puffing regime. And the numbers below each machine regime show the percent of average daily yield values on a per cigarette basis that fell below the machine-generated yields for the same cigarette when smoked in the study. And you can see that compared to the FTC or ISO regime, overall 19.4% of smokers had mouth-level tar exposure that was less than the FTC machine-generated yields. So said differently, more smokers yielded mouth-level tar exposure than

that produced by the FTC puffing regimen.

Conversely, focusing on the Health Canada puffing regime, 99% or almost all smokers had a mouth-level tar exposure that was less than that generated by the Canadian Intense puffing regime on a machine.

This next figure shows the number of cigarettes smoked per day from a mouth-level exposure study. On the x-axis we have the data for four subjects, and cigarettes per day was assessed on four different days, Day 1, Day 2, Day 15, and Day 16, over the study. All of these four subjects smoked the same brand of cigarette, and the noteworthy point is that, for any one subject, the number of cigarettes smoked per day was not constant. People smoked a different number of cigarettes on each day.

This next figure shows the per cigarette mouth-level tar exposure. This data is from a single smoker. On the y-axis you have the mouth-level tar exposure in units of milligrams per cigarette, and each data point on the graph or on the figure represents the tar yield achieved by the smoker when smoking a single usual brand cigarette on each of four different days. And you can look at any of the four different days, but you can see that the per cigarette mouth-level tar

exposure varied quite significantly from cigarette to cigarette. So said differently, even though this smoker smoked the same brand of cigarettes, mouth-level tar exposure was not constant.

This next figure shows the daily mouth-level tar exposure, and again for the same four subjects that we talked about just before on the cigarettes per day figure. Daily mouth-level tar exposure is on the y-axis in units of milligrams, and we have the four subjects with a daily mouth-level tar exposure shown on each of the four different days. And again, a key point to focus in on is that, for any one subject, daily mouth-level tar exposure was highly variable.

A final figure for mouth-level tar exposure studies: This represents data from studies conducted between 2007 and 2013. On the y-axis we have the daily average mouth-level tar exposure per subject in milligrams per cigarette. Each data point represents that value for a total of 2,700 smokers, and that daily or per cigarette mouth-level tar exposure was graphed against the FTC or ISO tar yield for that cigarette as generated by machine smoking. And you can see that regardless of the FTC or ISO tar yield, the mouth-level tar exposure per subject varied by as much as tenfold.

So, in summary, it should be evident that any one individual smokes their usual brand of cigarette in a highly variable manner, and similarly across smokers, daily mouth-level tar exposure also varies. Thus, a principal determinant of mainstream smoke exposure and toxicant exposure is an individual's smoking behavior.

And the evidence overall suggests that the extent of variation that occurs when a smoker smokes their usual brand of cigarettes far exceeds the differences in yields observed for two products of similar design when smoked by a machine.

Just very briefly I want to share this data, but mouth-level exposure has been estimated also for smokeless tobacco users. Similar to cigarettes, a variety of constituents have been measured. And again, similar to the data I just showed you on cigarettes, reported values for smokeless products show significant variability within the same individual and across individuals.

So, finally, I'm just going to talk very briefly about biomarkers of exposure. These are biomarkers that are certainly attractive to assess exposure, since it offers the potential to estimate constituent uptake based on actual use behavior.

There are, however, certain considerations or challenges associated with biomarkers of exposure. Firstly, you have to consider whether the biomarkers of exposure are relevant to disease risk in humans, and what relationship is there between the biomarker of exposure and actual toxicant dose.

In addition, you need to consider the degree of metabolic polymorphism that exists in any given population. That may give rise to different biomarker measurements in bodily fluids, even though the exposure may be the same.

And finally, when conducting cross-category comparisons, cigarettes to smokeless products, for example, you need to consider varying metabolism depending on the route of exposure, inhalation versus oral.

So just a couple of slides showing how biomarkers of exposure data can be used. Dr. Boffetta mentioned the NHANES database. This particular data was pulled from the biomonitoring data contained within NHANES. The data shows urinary cadmium levels in three groups of people: cigarette smokers, smokeless tobacco users, and nontobacco users. And we can see that when adjusted to gram of creatinine, the levels of urinary cadmium were significantly higher for cigarette smokers relative to smokeless users and nonusers: 0.44 µg of cadmium

per gram of creatinine compared to 0.25 in both smokeless users and nontobacco users.

Furthermore, that data was then correlated with serum cotinine, serum cotinine being a measure of tobacco product usage for those three groups of individuals. And among the cigarette smokers, serum cotinine was significantly correlated with the urinary cadmium results, again supporting the view that the increase in urinary cadmium for smokers was as a consequence of tobacco product exposure.

Among the smokeless users, serum cotinine was not associated with urinary cadmium, indicating that whatever cadmium the smokeless tobacco users were exposed to was not from the tobacco product itself. And finally, those urinary cadmium concentrations were compared to a biomonitoring equivalent value, which is the concentration of cadmium in urine as consistent with U.S. EPA's established reference dose, the reference dose being the dose that would be considered to have no adverse non-cancer health effects.

And overall and consistent with the risk profile between cigarettes and smokeless users, more cigarette smokers exceeded the biomonitoring equivalent compared to smokeless users and nontobacco users.

And so finally, in conclusion, various methods are available to characterize exposure to constituents associated with different types of tobacco products. Measurements of exposure in humans underscores the variability that we see in tobacco product use behavior.

And finally, any exposure assessment that is conducted needs to consider the product characteristics and evidentiary requirements of the regulatory pathway. For example, in substantial equivalence where by design you're comparing two products that are inherently similar, machine-based measurements should be sufficient to compare those products, in particular when considered against the backdrop of human use variability. Conversely, however, for modified risk tobacco product applications, exposure metrics in humans to support a claim of reduced exposure or reduced risk will be required, particularly for products of inherently different design relative to combustibles, for example.

And that's it. I finished quick.

(Applause.)

DR. DRESLER: Okay, it is break time. So a 15-minute break. We will reconvene at 20 until 3:00, so please be back on time, and we'll go ahead and complete two more speakers and

then the panel, okay? So 20 until 3:00.

Thank you.

(Off the record at 2:24 p.m.)

(On the record at 2:40 p.m.)

DR. DRESLER: Okay. All right, we'll get ready to start again. We have the two speakers, and then we'll have the panel. Our next speaker in this session is Dr. Bahman Asgharian from Applied Research Associates, speaking on the Use of Dosimetry Models in Predicting Internal Dose: Implications for Risk Assessment.

DR. ASGHARIAN: Thank you very much. First, I would like to thank the FDA for giving me the opportunity to speak here today. I'd like to start my presentation now. Yeah, I'll be talking about the prediction of internal dose as a result of exposure to cigarette smoke. And first -- this is not moving. Oh, okay, I was going in the wrong direction. Sorry.

Yeah, first, I'll give an overview of what I will be talking about. I'll be talking about challenges in characterizing tobacco risk, and this is just in the context of dosimetry modeling, not other factors involved. Then I will talk about dosimetry modeling for tobacco products, and I'll be talking about the inter-subject variability and exposure. Then

I'll talk about the state of the modeling and what is the latest -- currently what we have available, what are the shortcomings and the limitation if you want to use it for tobacco products. And then I'll give two examples to show how a dosimetry model can help with demonstrating uncertainty due to inter-subject variability and exposure.

The first example will be on internal dose as a function of age. I'll be comparing deposition of the particles in the lungs of children versus adults. And the second example would be on exposure to tobacco products in particular. And then I'll conclude and sum up.

Before I talk about challenges, I thought I should show you a simple diagram to explain why the internal dose estimation is such an important part of risk assessment for tobacco products, and especially after the few talks that tend to ignore internal dose, and I think that's something that needs to be considered.

Internal dose actually is the bridge between exposure environment and body response. As shown in this simple diagram, I show you that it's an intermediate step to calculate risk. So if you assume that risk is exposure just on concentration, you are ignoring mainly the inter-subject

variability, meaning that if people are exposed to the same exposure, they're going to have the same response, but that is not the case.

So with the internal dose, we can account for variability in exposure and, as I mentioned, differences in geometry and ventilation and so forth. So it helps. If you don't include that, if you don't use that, you're going to introduce uncertainty into the risk model, and when that happens, then you have a risk plot to include the uncertainty factor, which many times is just a random number. So internal dose is a major part of the risk assessment.

So what are the challenges in characterizing tobacco risks? All right, we'll turn to two actually. They are exposure risk challenges, and then there are smoker challenges. And again, I'm just talking about in the context of the dosimetry modeling.

First, there are a large number of potentially toxic constituents in tobacco products, and that goes for both combustible and electronic cigarettes. We need to identify these and account for them in the model.

Second, the mouth environment itself has an impact on the exposure of the smoke that is inhaled. The smoke in the mouth

undergoes changes. There's deposition due to aerodynamic properties of the particles, and there is constant phase change due to various reasons. So that is a challenge that we need to account for and because mouth environment impacts directly to deposition in the lung.

The third, we have to consider the behavior of this puff that is inhaled, and that includes for both the particles and vapor, which together form a very complex mixture. And an earlier presenter talked about that, that this mixture undergoes various changes. There's going to be hygroscopic effects. There's going to be phase change, meaning it goes from particles to vapor phase. There's going to be coagulation and so forth, and I'll talk about that a little bit later.

If you switch to the smoker, there's a variation in the deposition efficiency associated with age, gender, physiological status, which all together are called the inter-subject variability. So no two subjects have the same internal dose when they're exposed to the same exposure.

And lastly, there's variability in innate sensitivity due to specific constituents.

So the health effects are a function of all of these parameters that are listed here. I will be talking about the

first bullet, and in essence, there's a different talk, which I will not get into.

So I'll talk a little bit more about deposition modeling. Deposition modeling has been around for a long time. There are lots of models out there. Currently, most deposition models have been developed for environmental inert aerosols, and they're pretty much okay for using it in that context, for a typical environment or application, I called it. But for cigarette smoke, that is not the case because there's a challenge with the combustible and electronic particles because, as I said, they're very volatile, there's active phase change and so forth. So these models are not able to account for that, so there's a major disadvantage if you try to use the current models to predict the dose of smoke. What will happen, the dose obviously will be predicted incorrectly, so that will introduce a significant uncertainty into the risk model.

So where we need to develop a deposition model is specific to cigarette puff, and that also obviously improves prediction but also allows us to be able to predict not just the deposition for the entire puff but for the constituents. And that really helps with the risk assessment. So improved prediction for deposition of constituents supports more

accurate prediction of the risk, and this will provide the decision maker with information for risk management and so forth.

You may have heard of or seen the multiple-path dosimetry model. It was developed mainly for environmental aerosols, and there's a version, of course, for gas. So it can predict gas and particles but not the mixtures, such as in the case of cigarette particles, because of the complexity that we know. So if we use the model, we will be under-predicting the dose, and I will show that in a second.

So here I have compiled data on the deposition fraction of particles and the cigarette particles from various studies. The left panel, the left plot actually shows the deposition in total deposition and in total deposition in the mouth. The blue bars here are the total deposition for different studies, and the red bar is mouth deposition.

So if you look closely, total deposition on average varies 60 to 80%, and for the mouth deposition we're talking about 20 to perhaps 60%. And for the alveolar region, we have another study by McAughey et al. It gives approximately 60% deposition fraction for low tar and medium tar.

So now if you use MPPD, this is what we will get. For

total deposition, you only get about 20, maybe 20 to 30 if your particles are too small. And for the mouth deposition you only get a few percent. So there's a huge discrepancy, so that justifies the need for a more elaborate model. So basically, existing models significantly under-predict deposition, and we need to develop a dosimetry model just for cigarettes.

So the first step in developing the model, we need to characterize the puff, knowing that the puff is a very complex, unstable mixture and is quite different from environmental aerosol. So, first of all, once it's generated, we are talking about 10 to the 9 particles per cc, a very high number of concentrations that undergo very rapid coagulation.

And the mixture is a combination of vapors and particles, and it can -- I guess the number maybe is just an approximation, but in the combustible cigarette, you are talking about 5-, 6,000 particles -- I'm sorry, constituents, and for electronics, at least five and more.

So another thing about this is that these constituents have different saturation vapor pressure. So what that means is that the ones with low vapor pressure stay in the particulate phase, being the droplet but different constituents. The ones with high vapor pressure will stay in

the gaseous phase. And the one in the medium vapor pressure are the ones that undergo the phase change. There will be constant transfer of vapor to droplets and back and forth, depending on the conditions you're under.

So the behavior of this mixture of puff is influenced by these, and these can be categorized into two, colligative and non-colligative effects, which I will talk about shortly.

First, I'll talk about non-colligative effects. Non-colligative effects actually are shared by many particle types, and it's fully common. It's referred to -- coagulation and phase change. Coagulation, which is for high concentration of particles, is just simply the collision of particles in the air due to the thermal energy, and when that happens, particles grow in size and reduce the number concentration. And this really depends on the concentration of the particles, the size of the particles, and temperature of the particles.

The second mechanism is phase change, which is significant for medium vapor pressure and constituents, and it's simply just the evaporation and condensation of semi-volatile components. And similar to coagulation, the rate of size change depends on the initial particle size, but it also depends on the diffusion rate of the vapor component and the

amount of vapor in the air. So when you don't have any vapor in the air, the evaporation is much faster. But as the concentration of vapor builds up in the air, that sort of plays like a barrier, and the evaporation becomes more difficult.

The second mechanism for the behavior of the puff is the colligative effects, which is a fairly new, perhaps, concept and unique to cigarette particles. This colligative effect is for highly dense mixtures and is essentially the hydrodynamic interaction of particles with each other when in the gas phase.

So to basically describe what it is, I have these little diagrams here. So you can assume first -- I mean, these figures are exaggerated. Assume there are particles in the air, but they're far apart, they don't see each other, so air is flowing through them. So they are so small and far part that air will not see them and just simply flows through them.

Now, if you bring the particles closer to each other, the space between them gets constricted. So some of the air flows through them, and some goes around them. And when they are too tight, when they are closely packed, there's no space in between them for the air to go through. So basically all of the air is just diverted around them.

So in Case 1, we don't have any colligative effect, and

Case 3, we have a highly strong colligative effect. And the second diagram is in between this. So when the particles are closely packed, you have a strong, high colligative effect. They behave as a single particle, and they have a distinct size, density, and viscosity. And now they appear bigger, so they will have a reduced hydrodynamic drag force, and they travel faster, and that explains why they deposit more. So that's the mechanism for cigarette particles depositing more than normal.

So the approach for deposition modeling, I will not go into any detailed mathematical modeling, perhaps it's not of interest here, and just mention that what we need to do is to include in the existing models the mechanisms that are specific to cigarette particles and then be able to use that for predictions of the deposition of particles.

So basically what we have to calculate is deposition fraction during a puff inhalation scenario. And for that, we need to give the model the input parameters such as exposure parameters, which are concentration, size distribution, or lung geometry parameters. We need to have there, of course, some geometry of the lung and what's the lung volume, what's the FRC/TLC total volume, all the volumes we need, and also the

subject's breathing rate and breathing profile. With that, you are able to calculate how much particles deposit in the lung.

And given that the lung geometry is very complex and also the breathing itself is very complex, we have to make simplifying assumptions to develop the model. So I've here listed the major assumptions that we have made.

First, for the lung geometry, we just assume that a lung is basically a dichotomous branching structure, and each airway is represented by a single tube. And for the lung ventilation, the flow into the lung is due to a pressure drop with outside air and the pressure in the flow cavity, and it's not a push -- you know, I guess we have seen some of the studies, and it's really unrealistic.

So the pressure drop: The flow rate has three components. There's three pressure drop effects: the lung compliance, airway resistance, and flow inertance. In reality, all of these are cut up, but for us to be able to simplify, we assume these are independent mechanisms, so we have a simplified model, and this is pretty typical in the field of physiology. So we haven't really invented anything there, but we're just applying it here.

So now I will not talk about the deposition model, but

what I will talk about is how are we going to extend that, or what scenario we are going to simulate in the model.

So basically what we have to do is to simulate an inhalation puff, which is inhalation plus exhalation. So we started a puff that is generated. It is inhaled into the alveolar cavity. Its geometry is actually the alveolar cavity. We constructed that on the computer.

So we had a puff withdraw first. During puff withdraw, particles are deposited by inertial impaction if they grow and they have high velocity, but diffusion for the smaller particles. And particles may have much higher temperature than the body, so we also have thermophoresis.

At the end of puff withdraw, we have mouth-hold, and during mouth-hold, we have additional deposition by sedimentation, gravitational settling, and Brownian diffusion. And then we have dilution. Diluted air is inhaled and mixes with the puff, and that mixture will travel into the lung. It first reaches the tracheobronchial region where you will have deposition by inertial impaction and Brownian diffusion for large and small particles. And the reason for that, of course, is that we have a high flow rate into the lung, so impaction is significant.

So the fraction that doesn't deposit in the tracheobronchial region gets into the pulmonary region, and there, now the flow has dropped, the flow rate has dropped substantially because the number of airways have increased. So we have deposition by sedimentation for large particles and diffusion for small particles. And then at the end of this inhalation, we have mouth -- lung-hold and then exhalation. So that is the scenario we are simulating basically and putting into the model.

So now I move on to two examples that I talked about. The first example is the calculation of internal dose as a function of age, and here age is significant basically because we know teenagers smoke. I mean, I will show you results on children, but actually that was just to show the point. But what we need to focus is more on the teenagers that start smoking. So what's the lung dose compared to the adults?

So first we need to know a few things about the lung geometry of children. Although the geometry of the lung and physiology of children is quite different from adults, it's not simply a miniature version of adults because the growth rate is highly nonlinear. At birth, the tracheobronchial region is fully developed, but the alveolar space is not. It takes about

8 to 9 years for the alveolar space to be completed, and then after that there's a growth and still, you know, it has to go through puberty. It's still nonlinear.

The other thing to consider is that the building parameters and activity patterns are different between children and adults. So as a result, the delivered and deposited dose of inhaled smoke may be substantially different between children and adults. So therefore we need an age-specific model to conclude the geometry and physiology of children. And if we need a model for geometry, it's quite possible we need a model for the risk assessment specific to children as well.

So there has been some modeling for children, but early models are based on assumptions and simply because data were not available. I mean, the first assumption was that the lung, assumed to be just a miniature version of adult lung. So by that, what I mean was that they took the adult's lung and scaled it as a function of -- a logistic function of weight or height and assumed that would be the lung of the children. The other assumption was that there was no data had deposition. They just simply used the model that was available for adults. So the model actually was very unrealistic. And, in addition, the models were crude; there were many compartments. It wasn't

physiological, just compartments for different measures.

So as a result, we thought this was not sufficient, so we came up with a new generation of deposition model. So we took advantage of the data that are recently available for the head, there are deposition data in the head, so we came up with a semi-empirical formula that is able to predict the dose in the head region.

And also for the lung, there are measurements of the lung of children from ages of 3 months to 21 years old. So we use the data and construct the geometry as the children grow to become adults, the same thing with the physiology. So we put all of this together and develop lung geometry for -- a deposition model for children.

So I'm going to show some results here. Actually, I'm just looking -- right now I'm just looking at the environmental aerosols. So we plotted or calculated deposition fraction as a function of particle size, very small, 0.01 to 10 microns, and we calculated it for different lung lobes. So we see a typical behavior for the lung. There is a high deposition in the upper lobes -- I'm sorry, lower lobes and left and right lobe was higher, the middle lower lobes higher and the upper lobe lower than the right middle lobes.

I just looked at the time, and I lost my train of thought. So I don't have much time left, I guess. I'll have to speed up a little bit here.

So basically what you saw is that you can adjust your deposition with volume, and that's what we did. So we wanted to get that. We saw that we got a single curve for all of the deposition. And we also plotted deposition fraction as a function of total deposition, and that there was a plus. And looking at this, now it gives us an idea because, for each age group, you can come up with a single deposition curve. So that gives you a means to go across ages.

So what we did next was we looked at deposition fraction for different ages. Again, we don't see any relationship here, but once we adjust the volume, we see a clear pattern. Here, the deposition of particles is greatest -- adjusted deposition, not deposition -- that as the child gets -- as people grow, deposition decreases. So that actual adjusted deposition fraction is a dose metric for risk assessment.

We can do the same thing with the scaling factor. It could be for through unit surface area. And that, we can see that the same pattern appears but is not as clear as we expected though. So basically, we have a dose metric for risk

assessment.

So let's go on to the second example, and I have to move -- I'm sorry, I have to move up a little faster here. So the second one is that we simulated breathing scenario. We assumed a subject with a 50 mL puff, and he holds in oral cavity for 2 to 3 seconds and then inhaled the dilution air orally or oronasally.

And then for the puff, we just -- you've got thousands of constituents that are put into four groups: water, nicotine, which would be non-protonated, meaning that it's really available for evaporation and condensation and so forth, semi-volatile and soluble. So these are the scenarios.

So now look at the particle growth. This is kind of a medium, meaning there is no vapor around it. So the particles then grow freely, and they grow very fast, depending on the initial size -- the final size.

So we looked at the constituents in the puff. What you see, we looked at two cases, in the oral cavity and the lung. In the oral cavity, because the air gets saturated quickly, we don't even see much changes here or different constituents, but the nicotine in the lung evaporates quickly because we are assuming there is no vapor around it. And the particle size

grows actively due to hygroscopic growth basically.

Look at oral deposition during puff withdrawal and mouth-hold. And I will not get into details, but this curve -- is for non-colligative effect. Basically, it is a model similar to environmental aerosols, and we can see that deposition fractions are underestimated, very small. We have a 10 to 3 here. So it's very small at 0.003, which is unrealistic. So what happens if you include colligative effects? Now, the predictions become much closer to what is expected, the measurements.

So now look at lung deposition in the lung. So you're looking at the no mixing, mixing with and without colligative effects.

So in just enough time, I'll just give you the take-home message, is that if you look at the dashed line, which is the non-colligative effect, it's much lower in the TB region. But colligative effect increases deposition in the tracheobronchial region. But in the alveolar region, they are both pretty much similar, and the reason for that obviously is that because by the time you get to the lung, the puff has dispersed, the colligative effect has been reduced tremendously.

And the case of no mixing gives you much higher deposition

than complete mixing, and that's because when you inhale pollution air and mix it with the puff, you already diluted the thing, and there's no colligative effect.

So that brings me to the conclusions and the take-home message, which I don't know if I have time. Maybe you could --

(Off microphone comment.)

DR. ASGHARIAN: Okay. So basically, the idea of what I went through a little fast at the beginning, and it took my time, it explains that you can show these two examples that the simpler model tends to underestimate exposure and risk, but this uncertainty can be reduced if you use a detailed dosimetry model for cigarette particles. And the approach that we are recommending accounts for the constituents in the puff and can predict across ages.

And one other, I think, important thing is the dosimetry model based on the constituents, specific constituents, is an important starting point to evaluate additive effects of multiple constituents.

And then, finally, the reliable predictions of internal dose supports comparison of risks across different tobacco products and has the potential for rapid regulatory determination.

With that, I conclude my talk. And thank you. And sorry for going over time.

(Applause.)

DR. DRESLER: Okay, our next speaker and the last speaker for this session before our panel is Dr. George Woodall from the U.S. EPA. He'll be speaking on Exposure Response Analysis and Comparing Among Existing Health Effect Values.

George.

DR. WOODALL: All right. Well, I'm going to switch gears for you a bit. I'm going to do a little bit of back-casting and a little bit of forecasting into the talks that will be happening tomorrow and some today. There are a number of tools that we've developed in our risk assessments that we do for NCEA. We developed the IRIS assessment, the RfCs and RfDs, and I'll talk a little bit about those. Also we developed cancer slope factors and inhalation unit risk for cancer risk. And then we also developed the Integrated Science Assessments for the NAAQS.

And, of course, since I am with a government agency, there is the ever-present disclaimer that I'm not speaking for anybody except for myself.

Okay, so a quick outline: I'm going to go very quickly

over the risk assessment paradigm because that's been well covered by speakers before, go a little bit over reference values and risk estimates, I'll talk a little bit about those, the context of how they're used in risk assessment, and a brief intro into reference value arrays, which may be a useful tool in this context as well.

Then I'll talk about exposure response analysis and exposure response arrays. And I think that's the reason Phil wanted me to come and talk to you today. So it's the main topic, and I'm going to focus mostly on that, and it is a tool to compare and analyze endpoints and across durations. If you hadn't guessed, I was the one who asked that question in the earlier session. And I'll also talk about some of the future directions that we're looking to go into in that type of analysis.

Hazard versus risk, hazard and risk. Hazard is the source, and risk is the chance or probability of that hazard causing an adverse outcome.

And for risks to occur, you have to have a hazard first. You also have to have that exposure. This is very simple, and this is again another representation of the risk analysis or risk assessment paradigm, and we're going to be talking in this

area. And here in particular, we're talking about exposure assessment, but it eventually leads to a risk characterization, and that's where reference values come in handy.

Okay, reference value: This is my own definition, and other people have different definitions. There are a number of synonyms that are out there for reference values: toxicity values, health effect values, health benchmarks. There are a lot of different terms that are used to basically describe the same thing. The reference value is a general term that I use for a specific agent. Usually -- it's not usually for mixtures, designed to specify a level for adverse health effects. Agents can be chemical, microbial, or radiation. Health effects can be cancer or non-cancer. Some examples that are in the IRIS program -- and probably Vince will be talking, Vince Cogliano will be talking a lot about that in his presentation tomorrow. For cancer, you have oral slope factors or inhalation unit risk. For non-cancer, you have oral reference doses or inhalation reference concentrations.

Now, the relevance to the risk assessment paradigm, that is part of the dose-response assessment, and that when combined with the exposure assessment, you come up with the reference values in the dose-response assessment. When combined with the

exposure assessment, that then leads to the risk characterization.

So that's what you would relay to someone who's trying to make a decision about what that risk is, what is an acceptable risk level, what are the other cost-benefit determinations, because again a lot of these decisions are not made in a vacuum. There are all kinds of economic, political, social consequences to a lot of these decisions, but they're made in the context of EPA regulatory programs. And that's where I live, so that's kind of where I come at these things.

So, again, some more about health effect reference values. There are different types. Many of them are associated with different types of exposure scenarios, and I'm going to talk a little bit about that, too.

Public health values: An example is the RfC, which I mentioned before.

There are emergency response values, the acute exposure guideline levels, which again are for the general public, but they were designed for a once-in-a-lifetime exposure scenario, not repeated episodic exposures. That's not how they were designed. That's often how they're used, but that's not how they were designed.

And then you have the occupational values. TLVs is an example of threshold limit values, and they are designed to protect workers, so not for the general population.

I mentioned briefly some of the different categories: emergency response, occupational, and general public durations. These categories were developed by the Risk Assessment Forum of EPA: acute, less than 24 hours; short-term, 1 to 30 days; sub-chronic, over 30 days up to several years; and then chronic, up to a lifetime. And let me move on.

And I am very interested in this concept here. When we develop an RfC, it is basically an exposure limit for a lifetime. So you can have this average exposure, and you're still -- this lighter line below here is the lifetime average exposure, but you have multiple events that happen during your lifetime.

Do any of these peaks cause an effect that might not be protected against? In the occupational exposure paradigm, they have time-weighted averages, but they also have ceiling values, so that if you are exposed for a shorter period of time to a value that might not show up in that 8-hour average and it still has an effect, you're also being protected from that ceiling level.

I think this is very relevant, perhaps. I'm not really all that familiar with all the toxicology with cigarette smoke, but I mean, it's a short-term event. It might happen a couple times a day, but does that average, does that area under the curve actually protect you, or is it those peak exposures that are causing an effect?

So the tool, the reference value arrays: We have developed these for 29 different chemicals. A number of them are -- and I'll show that in the next slide -- chemical warfare agents. Actually, there was some work that I was being -- that I was doing with the Department of Homeland Security, and it actually led to this development of this tool, and it shows values that are for emergency response. Here you see the AEGLs and the emergency response plan and guidelines.

The occupational values: Most of these are on shorter times, but I show these going out to a 40-year career also.

And then you have the general public values, which are typically here in green.

And then also, here I use benzene as an example. You can also show the inhalation unit risk values. This right here is the range. There are two values that are an upper and lower bound for the range for cancer, for benzene, and this shows two

different risk levels. First of all, 10 to the -6, a 1 in 1 million potential or risk for cancer, is in this lower band, 1 in 10,000 is up here, and these are the ranges within each one of those.

So you can demonstrate -- you can show a whole lot of these different types of values, again, developed for different scenarios, developed for different durations, and you need to know a little bit about what went into the derivation. Whenever you have a graphic like that, you always have to have a table with details because a lot of those details just get glossed over.

So in this, we have the values themselves, the health effect, the point of departure that was used for that, what type of point of departure it was, the principal study, and the uncertainty factors and any different derivations that were used, like extrapolation for time, whether there were adjustments for occupational versus normal general public breathing rates, etc. And we also have a link to each of the sources, too. And this is just excerpts showing the general public values.

So there are a number of chemicals with reference value arrays. The ones that are in red bold here are ones that are

on the list that was provided. These are constituents that have been identified in chemical -- or in tobacco smoke or in tobacco products. The ones that are in asterisks indicate a chemical warfare agent, and you'll see that there are some where you have an asterisk, too. There may be another treasure trove of data for you guys to use if you haven't already taken -- availed yourself of some of the data that the Army has. Just up the road at Aberdeen Proving Ground, they've got a treasure trove of data on these chemical warfare agents and for a lot of these other things, too, that are not warfare agents but are toxic industrial chemicals. So they are targets of opportunity that they were concerned with.

So now I'm going to talk to the main topic, exposure response rates. First of all, what they're not. They're not gene arrays, proteomic arrays, or any of these heat map kind of things that you see. However, it does not exclude those types of data. Those types of data can be shown. And again, I've talked about reference arrays. Unfortunately they're both called arrays, so it can sometimes lead to some confusion.

So let me just talk about what it is. It's a graphical depiction of chemical-specific data, dose-response data, if you will. What we have here, if I'll give you the quick tour,

along the y-axis we have the concentration, and this is in a logarithmic scale, so we can show a whole lot of different data together. You then have different types of effects. We've also segregated data from mouse, rat, and then two different time frames, sub-chronic and chronic. So you can compare across a whole lot of different durations, different types of effects, and then also by different species. Each one of these are called an element, or I call them an element, and they show all of the doses that have been tested in this particular study. It identifies if it's a NOAEL or LOAEL or if there was a benchmark dose analysis that was done on those data.

So there's always supporting data that you need for any kind of array like this, as I mentioned before. It includes important data that you can't show graphically, and this is just an example of some of the types of information: the population of the animals, the species, a little bit more about the critical effects. There are a number of other things that can be incorporated into a table like this.

Now, there's always more information that you find from studies where you have a full range, a number of doses, so you have both above the LOAEL and below the NOAEL so that you know that you've captured what the full range is, a little bit less

information if you have an effect level but you don't know how low you have to go, or you have a level and you didn't find an effect but you don't know how much higher you have to go to see an adverse effect. And, of course, the single point is almost always least informative. It still provides information, but it's still the least informative.

So what not to do: This is a summary of a number of effects, neurological effects that are shown with lead exposure. There's a lot of data with lead. So I don't think anybody could read this. It's kind of hard to even -- you get kind of cross-eyed just looking at it.

So we've developed overviews or summary arrays that take and represent multiple studies and related effects all together. So this is collapsing a whole lot of information all together, where we have clumped these together: immune effects, neurological effects, renal effects, and reproductive effects. The other one was just neurological effects, so it was just a summary of this group here. So imagine if everything was on that graph, it would be even worse.

So at any rate, you can now compare, among these different classes of effects, which ones are showing up at the lowest exposure levels. This is all human data, so we don't have to

do any kind of adjustments. If you were using animal data, of course you'd have to use, as mentioned before, the human equivalent concentration adjustments so that you are getting to -- you're comparing apples to apples and not just making fruit salad.

So at any rate, you can look across all of these, and you can find the types of effects that are probably going to be driving your risk assessment, if again you are trying to protect the general population and, in particular, susceptible individuals.

So now we take that one category of neurotoxicity, and we have -- we're refining that summary array. Again, this is still an aggregation of a whole bunch of studies and each one of these elements. So it's showing the lowest LOAEL. I don't show the highest NOAEL because the highest NOAEL may be higher than the lowest LOAEL if you're grouping a whole bunch of studies. So I chose to use just the lowest LOAEL in this demonstration, so again looking across a number of different types of effects within that category of neurological effects.

And you can again find the effect that is showing up. This is effects in the neurotransmitters, but again, is that an adverse effect? These are questions that you need to ask.

These are the types of -- these types of displays are used when you're trying to evaluate the studies and trying to determine what is going to go forward with dose-response analysis. So you're not there yet. This is still in part of the screening, the systematic review, reviewed literature, and you get the studies that you say you're going to evaluate, and this is a tool to help evaluate those studies.

Okay. There is training available. We developed a course that's based on the risk assessment training and experience program that is available through RATE. We do have a half-day workshop, at least it was not canceled last time I looked, at SRA, coming up in San Diego next month.

There is a best practices document. It's in clearance. It has guidance on customizing arrays. It shows early phase to identify the critical effects and principal studies in later phases. Again, this tool can also be used to communicate the choices that were used for reference value derivation.

And there is a user's manual also being developed. It's been developed associated with the training course, and it is in clearance as well.

So now let me switch gears a little bit. That's what we have. We've got an active research program to try to move to

the next level. We're trying to incorporate a whole lot of the Tox21 type of information and go from where we are now and use a framework so that we can look across life stages, across exposure durations, across levels of severity, and come up with another way of taking a look at even more depth, more dimensions in the data.

And this is our surface analysis, is the term that we're using. Again, it's a characterization of responses across duration and concentration. As you guessed from the question that I asked earlier, I think duration is an important component, those repeated episodic exposures versus the area under the curve. It's not the same for all chemicals, and it's not going to be the same for all exposure scenarios.

So what we're trying to do is create a context for evaluating assessment approaches so that we can look at those acute and episodic exposures and find out if they might be determinants for an actual endpoint, the adverse effect.

So, again, I think this is probably going to be a good bridge for using systems biology and computational models. A lot of the key events understood as part of pathogenesis can be incorporated into this, and that will help inform AOPs. And then it informs case studies on cost-benefit analysis. And

that's a big aspect in our -- for our regulatory programs. If you've got a number, that's fine, especially for -- you know, for the non-cancer effects we develop one number. Okay, it's assumed to be safe for everybody except the hyper-susceptible. So you know that if you're exposed to a level below that, you're fine.

But not everybody is exposed to levels below that. There are people who are going to be out there and are going to be exposed at levels above that. How much higher before you start seeing effects? That's something that we haven't really been doing up to this point. We can do it for cancer because we developed a probability slope, but we don't typically do it for non-cancer effects. We probably should be, and we are working towards that. And I see Dale shaking his head. Okay, so we are working towards that.

And then again, some of the aspects that I wanted to bring forward, the concentration-duration-response surface analysis and interpretation. And again this, I think, is an important aspect. We need to be able to address a whole lot of different exposure scenarios, not just the chronic 24 hours a day, 7 days a week for a lifetime. There are those variations in exposure.

We are again taking a case study approach to develop some

of these, and we are looking at different types of chemicals, we're looking at reactive gases, solvents, metals, and we're also looking at a number of different types of effects, developmental, neurological, cancer, a whole lot of different types of effects.

And again, we hope to be able to bridge that systems biology and bring a lot of that information into our risk assessment and into our types of analyses, understanding key events, as they are a part of pathogenesis and aid again in the application of mode of action and adverse outcome pathways and pull that mechanistic data into our risk assessment a little bit more fully.

And talking about our framework, we want to be able to collect data across multiple domains, life stage, exposure duration, across different organ systems and looking at severity of effects, cross-cutting analyses, which will lead to latency. And this is important. Latency is, a lot of you have probably heard, early exposure leading to lifetime effects. There's a lot of research that's been done recently that can have developmental -- even in utero exposures that lead to later obesity, obesogens, and potential for diabetes, a whole lot of other different types of effects that occur later in

life.

So it's a latent effect, and there's not really a whole lot of good tools for trying to identify that. That's one of the aspects that we're trying to look at. If anybody has any bright ideas on approaches, I've got ears to listen.

And then, also, another issue that Lynne brought up was recovery time and repair rates. We need to make sure that we understand what those are so that -- one of the things that I like in that, too, is if anybody has ever taken scuba diving classes and you have learned about those Navy dive tables, you learned that you can only go down to a certain depth for a certain amount of time, and then you have to decompress a lot of times for other types of chemicals. It might be the same type of approach that might be useful. We were exposed for 8 hours a day in a workplace at this level. Will we be fully recovered after a night of sleep, or do we need to have -- maybe the next day you do something else so that you don't reach that saturation point and end up having something that's an adverse effect. The bends is pretty obvious, some of the other things we're talking about not quite so obvious.

And then, of course, dose metrics, I have to mention that. I work with Andy Jerovic (ph.).

So what we're trying to do is get to a way that we can compare multiple dimensions and just not concentration and response, but also looking at exposure duration here. We could look at any other number of different z-axes that might be useful to apply, and it all depends on, again, what the data show you.

So just to kind of review: We talked a little bit about the risk assessment paradigm. It's not necessarily shifting, but it's definitely evolving with newer data types.

And the obvious risk requires both hazard and exposure reference values that are important tools, and reference value arrays may be a tool that might be of use in your types of assessments as well.

And then exposure response analysis and exposure response arrays, it's a tool for comparing across a dataset, and we're trying to incorporate multiple dimensions into those types of analyses so that we can do not just a two-dimensional but a three-dimensional type of approach to analyzing our data.

So with that, I'll close.

(Applause.)

DR. DRESLER: I'll ask the speakers to come up, please. And Dr. Klepeis, if you're on the phone -- Dr. Klepeis is still

there?

DR. KLEPEIS: Yes, I'm here.

DR. DRESLER: All right, great. Okay, so the same thing as before, if you want to ask some questions. And we do, we have a question or two, so just kind of raise your hand and you'll get a card, and they'll pick them up, and we'll go with questions.

Dr. Klepeis, if you'll clear your throat or jump in there because that way I'll know that you would like to ask a question, okay?

DR. KLEPEIS: Okay.

DR. DRESLER: So the first question. So this is a question about particle size. So if you proceed away from a source of particulate exposure, the questioner would expect that some particles will settle out, with the larger particles settling more quickly than smaller particles. And so this would lead to a shift in particle size distribution, with smaller particles becoming a larger proportion of the mixture with time and distance. Have you seen this, and have you looked at the particle size distributions for secondhand smoke?

So, Neil, this one might be for you or for anyone, but -- so over time, that you will have the larger particles -- time

and distance, you'll have larger particles settling out and so you'll have a larger proportion of smaller particles. Have you seen this?

DR. KLEPEIS: Yeah, we've measured quite a bit of sizes of data chambers, and you see, you know, the coagulation effect, deposition, the differential for particle size. You have the accumulation mode. You have, you know, right in the middle, 0.1, 0.2 microns, you have these small particles that can then deposit in the lungs as well. And so you do see that effect over time when particles start to deposit or get removed, the larger particles and the smaller particles as well, to turbulence or deposited on surfaces.

So yeah, in terms of distance, we haven't really studied that. Mixing in a space indoors is generally pretty rapid as well, but it's difficult, I think, to see any effects there. They're probably not too relevant. Outdoors as well, very rapid. I mean, the wind will bring in the turbulent air and mix with things very rapidly. So we haven't observed -- we haven't really looked for those kinds of effects over sort of the mixing regime.

DR. DRESLER: I'm going to follow up with another question that came in. So as time progresses, what factors may be used

to determine when secondhand smoke becomes thirdhand smoke?

DR. KLEPEIS: Well, in terms of deposition and then readmission from the surfaces or contact with the surfaces, you have -- you know, over time you have all the particles and chemicals that are then plating out onto different surfaces in the room, and you could have an equilibrium between, you know, chemicals casting off the walls and off the surfaces over time.

Then you have things just that build up and get, you know -- or become sort of entrained into the drywall and the carpet and stay there for long periods of time, and if people, kids, you know, get ahold of the dust or have saliva that pulls this stuff off the walls, then you have that thirdhand smoke. Thirdhand smoke is generally just the surface-borne material that can be directly transferred to a human boundary, or it can then be re-entrained into the air. So there's a lot of work going on, on that area and a lot of different chemicals are being looked at. So it's pretty complicated.

DR. DRESLER: Okay. All right, Dr. Potts, this one is for you. Please discuss the change in relative risk to individual users as they move away from consuming a cigarette with lower ISO/Canadian Intense toxicant deliveries to consuming a cigarette with higher ISO/Canadian Intense toxicant deliveries.

So discuss the change in relative risk to the individual as they move from lower ISO/Canadian Intense toxicant deliveries to consuming a cigarette with higher.

DR. POTTS: So I think the question is asking what would be the difference in risk for an individual switching from one cigarette with machine yields to another cigarette with higher machine yields? Is that the question?

DR. DRESLER: So the question is, is that when the individual users -- what is the change in the relative risk to individual users as they move from consuming a cigarette with lower ISO or CI toxicant deliveries to consuming a cigarette with higher? So what is the relative risk to the individual as they move from lower to higher?

DR. POTTS: If I understand the question correctly, so first, machine yields don't predict human exposure. And so I think as an individual switches from one cigarette to the next with different machine-smoking yields, then the exposure itself would depend on how an individual smokes that product.

DR. DRESLER: Because you were saying that there's so much individual variability, but would -- if a person goes from a low ISO/Canadian Intense cigarette to a high ISO/Canadian Intense, would that individual be smoking a higher relative

risk cigarette?

DR. POTTS: Well, again, it would depend on how the individual uses that product, how they smoke that product.

DR. DRESLER: So that's what the ruling is, the ruling issue would be the individual smoking parameter.

DR. POTTS: Right.

DR. DRESLER: Okay. Okay, Dr. Woodall, what criteria or approaches are used to group studies into a single array element in the exposure response array? So what criteria or approaches are used to group studies into a single array element in the exposure response array?

DR. WOODALL: Okay. Well, you want to group effects that are similar in nature. I think that some of the criteria, you'd have to have the similar dose metrics. I think that has to be the same so that you're comparing the exposures using the same units. I think that's key. And also the measure for the response needs to be the same.

I think beyond that, some of the study design characteristics would need to be considered. It would depend upon the data that you're trying to summarize. And with the lead example, we had a lot of different studies to work with, and so we used fairly rigorous criteria, and those three are

the main ones. And again, it would depend upon -- you have to look at the individual study characteristics to make sure that they are similar enough that you have confidence that they could be grouped together. And with anything like that, you have to document the decisions that you made, the criteria that you used.

DR. DRESLER: Transparently document.

DR. WOODALL: Exactly, yes.

DR. DRESLER: Okay. Dr. Klepeis, you discussed in detail the exposure measurement of particles. How may the exposure to the gas phase, including SVOCs or VOCs, contribute to the toxicity of secondhand smoke, and how could it be quantified?

DR. KLEPEIS: Yeah, I talked generally about the bulk materials and how we assess exposure, but you can look at it, I guess, via semi-volatiles or volatile organics in a similar methodology to look at those in the models. And the models will be more complicated as you incorporate dynamics of nicotine, you know, reacting with surfaces, as we talked about, reacting with other chemicals in the air. So it gets very involved when you want to look at other types of specific compounds. But there's a lot of work that has been done on that as well, so it's conceptually similar to what I presented.

DR. DRESLER: Anybody else want to address these?

(No response.)

DR. DRESLER: Nothing, okay. Dr. Bahman Asgharian, the deposition you modeled by age shows surprisingly large differences. Would these apply to the constituents of smoke in air or secondhand smoke as well as puffs? So the deposition you modeled by age show surprisingly large differences. Would these apply to constituents of smoke in air or secondhand smoke as well as puffs?

DR. ASGHARIAN: Well, actually, I didn't have time to talk about it, but that's the next step that we have to take. We haven't done that yet, but I suspect that we are going to see differences as well, the same proportion, but -- I mean the same pattern, but I'm not sure if the differences are closer to each other or they're broader, so -- but you have to do it first to really make a final statement of that.

DR. DRESLER: Okay, all right. Dr. Woodall, would exposure response arrays ever include evidence of interactions, even if only binary interactions? Is it, e.g., ATSDR?

DR. WOODALL: I think I can guess who asked that question.

(Laughter.)

DR. WOODALL: They haven't to this point, but I would love

to take on the challenge.

DR. DRESLER: Okay, everybody else want to take that -- that's okay. Okay, Dr. Asgharian, while complex models may provide more accurate estimates of exposure and simpler models may underestimate exposure, what approaches are used to balance the development of models to estimate exposures? So what approaches may be used to determine when a model is considered complete?

DR. ASGHARIAN: Well, the model is never complete, I guess. You just have to be consistent with measurements. And I'm not sure if I really have an answer to that question, you know, how to get the best answer. The thing is the question, I guess, is -- if you want to pose it differently, is if the level of complexity justifies the model.

And in this case I was trying to show that is the case, you need to have -- for cigarette particles, to have some detailed models that account for the physics and with dynamics in particular, phase change and all that. No, I didn't really talk about the physics, the mathematics. It's very complex. You know, I just went over it very quickly.

But for the cigarette particles, because the puff itself is so complex and undergoes such a rapid change constantly, not

just in the mouth, because as particles are deposited or vapors taken up in the lung, the thermodynamic states of constituents change, and you're going to get -- it could reveal some of the phenomenon. So for cigarette particles, I think you need to have this level of complexity. But other compounds, I'm not sure.

DR. DRESLER: Dr. Boffetta, and everyone, too, for this, what areas of research and method development further strengthen knowledge and approaches regarding tobacco product exposure assessment? So what are the areas of the research and method development that are needed?

DR. BOFFETTA: Well, that's quite a tough question. I mean, I think one aspect that is particularly weak is the issue of repeated measurements, so to have multiple measurements of the same groups of individuals or whatever, both at the short term, which would really assess the reliability of the measurement, but also on the longer term to investigate trajectories and switching patterns, etc.

There is surprisingly little information on those aspects, which are critical, I think, both to interpret the data that we have on a lot of different populations, but typically cross-sectional at one point in time, and also to assess the

trajectories, which is an important aspect of the toxicity or the risk, you know, of the individual setting. That's really the main issue, which is not so much methodological development but more a way where to put resources in terms of, you know, investing future studies or designing future studies or subsequent studies.

In terms of exposure assessment, I mean, obviously biomarkers have been used and have been proposed a lot, but they are not without problems themselves, both in terms of confounding and in terms of possible sources of bias in the way samples are collected or stored and comparability. So this will open a whole different sort of conversation on the validity of measurements based on biomarkers, but I think these would be the two main areas to address the question.

DR. DRESLER: Anyone else?

(No response.)

DR. DRESLER: This is a follow-up question for you probably, also. What types of data are available to characterize the exposure to smokeless tobacco products?

DR. BOFFETTA: Well, I think I described some of those, as I said, maybe a little bit quick, you know, because I was trying to cover a big territory. The amount of data is much

smaller compared to smoking products, but basically we use the same type of information, I mean, both administrative data, health surveys, and there are more and more now in some of the bigger cohorts. The problem is that, you know, for smokeless tobacco, given that the prevalence in most populations is relatively low, with exceptions, you know, but you need to really match larger populations to derive, you know, valid data. I mean, that's really the point. So individual studies tend to provide fairly unstable results compared to smoking, at least to the classic results that we know about smoking prevalence.

DR. POTTS: I think in terms of smokeless tobacco products, I didn't show any data in my presentation, but there have been mouth-level exposure studies conducted of smokeless products where you measure constituents in the smokeless product before use and then measure the same constituent end product after it's been used, and by subtraction you get a sense of mouth-level exposure. It's a fairly straightforward approach that can be done.

DR. DRESLER: Okay. Dr. Woodall, different organizations have different criteria for acute, short-term, sub-chronic, and chronic time periods. In reference value arrays, from one of

your slides, can you explain how you have considered the exposure time frames between different organizations?

DR. WOODALL: Sure. If you look at the detail on those exposure -- the reference value arrays, we do have the categories that were defined by the Risk Assessment Forum as the bins. But if another organization, let's say ATSDR, who defines acute as being from 1 day to 14 days, it's still represented the same, it's represented on that time frame, it overlaps those exposure durations that were defined by the Risk Assessment Forum.

So that's only a construct that is laid on there, but the actual -- when the data or when the reference values are plotted on that array, it's still using the same units that are, you know, numbers of days, number of months, etc.

One of the things I didn't point out on there, too, there's also a logarithmic scale in hours across the x-axis, so that you can show things at the very short durations, and they are spread out, and then you have the longer durations, and they are also on that same array. So it is a way to show all of those, that full spectrum of duration.

DR. DRESLER: Okay. I'm going to ask for 5 more minutes because I have several more questions.

So, Dr. Potts, can you go over new mouth-level exposures as measured in humans? Can you go over how mouth-level exposures are measured?

DR. POTTS: Yes, I talked briefly about smokeless products. That is pretty straightforward in concept. You measure the constituent in the smokeless product before it's used, and then you measure what's left after the product has been used, and by subtraction, that's your mouth-level exposure for smokeless products.

In cigarettes, cigarettes are smoked as they would be ordinarily, and then after consumption of the product, the filter or section of the filter is removed, and that's extracted and analyzed for tar and nicotine, for example. And then by measuring those two constituents, you can then establish, by correlation, mouth-level exposures to other constituents present in cigarette smoke. It's a well-published technique, so I think if anyone wants additional details, by all means, go online and find it.

DR. DRESLER: How about e-cigarettes?

DR. POTTS: E-cigarettes, I'm not aware of it being done with e-cigarettes. Today, I don't know how you would do that.

DR. DRESLER: Waterpipe?

DR. POTTS: The same answer.

DR. DRESLER: Okay.

DR. POTTS: I have nothing on waterpipe, but I don't know if you could.

DR. DRESLER: Okay, all right. Thank you.

Dr. Woodall, for exposure response arrays, negative controls show a zero dose. How do positive controls display?

DR. WOODALL: A good question. Actually, most of the data that we've looked at doesn't typically incorporate positive controls. I know one particular aspect, genotox studies always have a positive control. You would probably -- okay, I haven't done it up to this point, but I think it would probably show that as a separate element.

DR. DRESLER: Okay.

DR. WOODALL: So you would have that comparative, what your positive control is, and then you could do -- and I think that might be what you could probably use for the previous question, as I'm thinking about it aloud. If you would do, you know, kind of multiple components, you would probably want to see what individual -- the response rate for individual components was and then combined and then see where that leads. But we haven't done it up to this point, and we haven't

incorporated it into any of the arrays, but I think that that would probably be an approach that would be --

DR. DRESLER: Okay.

DR. WOODALL: -- that I would probably take.

DR. DRESLER: I think this is a follow-up one. For exposure response arrays, how are weight-of-evidence quality scores displayed?

DR. WOODALL: Aha, a very good question. Basically, we haven't done that. The weight of evidence would come at either before or after. It depends on whether or not it's in the early phase. The exposure response arrays are used when you're just looking at all of the studies that have met some quality criteria to be able to be compared.

It would help to identify those particular types of effects that might be occurring at lower concentrations or where you might want to take a look at those effects much more critically. But weight of evidence has not been incorporated into those. And again, for the later phase when you're looking at those studies or those types of effects that were -- on which reference values may have been generated, that would have been after the weight-of-evidence evaluation was done. So we haven't done it, and it would be an interesting topic to try to

tackle. So get some wheels turning.

DR. DRESLER: Which is what we're hoping for with this workshop, so good.

Dr. Asgharian, what are the parameters used to measure lung geometry?

(Off microphone response.)

DR. DRESLER: Could you push your red button, please? Thank you.

DR. ASGHARIAN: Well, I mean, the lung geometry we used was based on the Lovelace data that is from the '80s. It was called the phonebook, and it had, you know, measured the lung, certain pathways, the length, diameter, gravity angle, and orientation angle. With this information, you should be able to construct the entire -- the parts that are measured and therefore the missing parts. You use a statistical relationship to complete the lung, and the same thing with the alveolar region.

DR. DRESLER: All right. Now, I have some more questions, too, but if I don't end this session and you get a quick break, you're going to be here way later. So if you had a question and I didn't ask it, please grab one of the presenters.

And Dr. Klepeis, thank you very much. Speakers in the

room, thank you very much for both your presentations and for the panel.

We're going to take only a 10-minute break this time, okay? And we're going to start on time when you come back. So let's say at like 8 after 4:00.

(Applause.)

(Off the record at 3:57 p.m.)

(On the record at 4:07 p.m.)

DR. DRESLER: So for our Session 3, which is Population Susceptibility, and our first speaker will be Dr. Dale Hattis from Clark University, speaking on the Variability in Susceptibility — Challenges and Opportunities for Quantitative Risk Assessment from Human Data.

Dr. Hattis.

DR. HATTIS: Yes, you all deserve a hand of congratulations for enduring to this late hour. So for your special delight or torture, as the case may be --

(Laughter.)

DR. HATTIS: -- I have for you two probabilistic concepts, basically variability and uncertainty. I'm going to then show that -- because I'll be talking mainly about variability information, I need you to keep the variability and uncertainty

separate in your heads because otherwise you're going to be really confused. Then we're going to go into the importance of causal mechanisms and using variability data, in particular. We're going to talk about some specific issues in the use of epi data to estimate human population dose-response relationships, and then we're going to be showing a little bit of application to one kind of effect of cigarette smoking, which is the chronic loss of lung function.

So uncertainty and variability are two different concepts expressed in a language of probability. Variability means real differences among individual cases or people or categories in some parameter. Uncertainty means an imperfection in our knowledge of the true value of a parameter, either for an individual or for a group.

There are some underappreciated elements of variability. First, standard statistical descriptions of the data, for example, standard deviations, tend to overstate the real variability by including measurement errors. So, basically, you take your set of measurements, and then you take the standard deviation of those or the geometric standard deviation you have implicitly included in your measure of variability, measurement errors, and unfortunately it's only the real

variability without the measurement errors that affects the spread of individual risks, okay?

On the other hand, of course, unrepresentativeness of the sample is an important threat to the accuracy of any analysis of variability.

In priority setting, if you kind of set priorities among a group of candidates for intervention or for the use of your scarce agency resources, you want -- the more predictable variability there is among those categories that are candidates for your action, the more you are rewarded for using a priority setting system. How much relative bang for the buck do I get in this category versus that category?

Now, uncertainty also has some underappreciated features. Standard statistical description of data, say a standard error, right, that you knew, tend to understate real uncertainty because, calculated in the ways that they're standardly calculated, they don't know -- they're only based on random error among the measurements. They don't know anything about a systematic error that might affect all of the measurements that you have available.

So there are ways of trying to expand the expected uncertainty to compensate for that, but they are -- like the

variability problem, they're almost never used, okay? And I won't go into the details of how you cope with that, but suffice it to say that that's -- that it's true that your uncertainty is understated by standard measures of uncertainty, like standard errors.

Incomplete assessment of model errors is, of course, an important threat to the accuracy of an uncertainty assessment.

And in a priority setting system, in contrast to variability, if you have lots of uncertainty among the priority scores of a particular -- you know, of particular candidates for intervention, then you are better served by spreading out your resources among different candidates for a priority for intervention, because when you spread your resources out to lower-priority candidates, you get more information about what the real variability -- what's the real return on investment for those things. So it's basically they have very different implications for risk management priority setting.

I'm going to try to suggest to you that mechanism-driven and empirical modeling of variability distribution is important. Variability from the addition is influenced by mechanisms that produce the variability. Variability that arises by the addition of lots of random numbers implies a

normal distribution is a central limit theory of physics that shows that. But suffice it to say that if you're adding lots of independent influences that kicks it one way or another, of molecules dispersing from a source, you tend to get normal distributions.

Right, there we are. Variability from multiplying lots of random numbers -- and this is actually the most common mode of producing variability -- implies a lognormal distribution, that is, the logs of the parameters that they're varying tend to be normally distributed.

Okay. Variability from different forms of a gene, if they're the major influence on the parameter you're measuring, or other measured determinants of the behavior of an outcome parameter, implies a mixture of normal or lognormal distributions that you can also fit, but they tend to be more complicated and have more parameters than the simple, normal, or lognormal distributions. So they require a bit more data to be confident that you've got a good description of the phenomenon.

Let me talk to you a little bit about a scale for measuring lognormal variability. Very often pharmacokinetic variability is about 0.3, about the third line in that scale,

and that's the case for the variability that I'm going to quantify at the end, in individual FEV_1 responses to cigarette smoking. So what that means is that the 5th percentile to the 95th percentile spread of a lognormal distribution is about tenfold or so. The 1% to 99% spread is about -- more close to 25-fold.

It's also possible in individual cases to get much larger variability for specific kinds of effects, but I'm not going to go into -- basically, I've got a database of individual variability observations, mostly constructed from drugs and selected environmental chemicals. And so anybody who wants to know the distribution of expectation of random cases can consult that database, but I'm not going to tell you the details of that today.

To give you an idea of how these things are measured, this is empirical measurements of breathing rates in coal miners, and then you can see what's going on here; there's quite a bit of variability in this histogram presentation. Z = -1 is one standard deviation to the left of the distribution, to the low end of the breathing rates. Z = + 1 is one standard deviation above. And basically, you can convert lots of the information to this Z format.

And the advantage of this kind of format is that if you plot the data, it turns a curvilinear normal or lognormal distribution into a straight line, and that's convenient because basically then the intercept is an estimate of the mean of whatever you're plotting, and the slope is an estimate of the standard deviation, and so that gives you information from either truncated data or histogram-like data.

So in this case, we're fitting a normal distribution to the distribution of breathing rates observed in 60-odd British coal miners, and you can see that a normal distribution fits quite well. By contrast, a lognormal distribution fits not quite as well. I don't think one can really definitively conclude that this distribution is normal versus lognormal on this basis, but it's a little bit -- you can at least see from this that you can quickly get a sense that the normal distribution is a little bit better, just from the R-squared.

Dealing with common problems in the analysis of epidemiology is a key challenge to using variability information for risk analysis. The first problem that you have in dealing with epidemiological data is getting your hands on it. When you ask an experimental biologist or toxicologist for the underlying data that they published on, you will almost

always get it.

If you ask an epidemiologist for the same thing, you will almost always not get it, for one reason or another, some excuse about individual -- protecting individual information or whatever. Also, measurement errors are rarely quantified in ways that can be used to adjust for errors-in-variables biases.

Okay, if you have a dose-response relationship or -- sorry, ordinary least squares techniques for regression analysis handle well uncertainty in the Y direction. But if you have uncertainty in the X variable, that is, the exposure variable, it causes a distortion in the slope of the relationship that you derive, so that in fact you have a bias, a lower specific bias. If you have a linear relationship, you tend to underestimate the slope and overestimate the intercepts.

The other problem is that your real variability estimates are contaminated with this measurement imperfection. Dealing with measurement error in the simplest case, basically, if you have an estimate of the measurement variance, you can subtract that from the estimate of the total observed variance to get an estimate of the real variance, that is, assuming both the measurement error and the long-term variability are normally

distributed or lognormally distributed in the case of a lognormal distribution.

Unfortunately it's rare for the epidemiologist to make measurements, to disclose and making disclosed measurements of their dosimetry uncertainty, and that's a problem. You have to otherwise make some external estimate of whatever information you'd have from other studies.

Treatment of censored data, that is, you know, non-detects. This is actually fairly straightforwardly dealt with. The older, obsolete view is basically you assign, arbitrarily, zero for non-detects, and that's wrong obviously because there's something there. The current usual practice is to arbitrarily assume one-half of the detection limit, and that can be wrong as well, just because it depends on how many cases you have that are near the detection limit and how many that are pretty far from it.

The way you really should be handling this, I argue, is with an imputation technique. You basically take and draw -- do the linear regression to get a Z score versus the log of dose, for example, and then fit that kind of relationship to the data that you do have in the detected region, counting the Z score -- calculating the Z score from the full dataset, and

you can then estimate a central tendency value for the non-detects from the fitted distribution of the rest of the data. This is not a careful renovation, but it's fairly straightforward to actually do this and, I think, should be preferred over the common practice of using one-half the detection limit.

I want to suggest to you that dealing with variability information is different, depending upon what kind of toxicological mechanism you think is happening to cause the adverse effects. And basically this comes out of a fourfold system of classification of adverse effects that have different implications. One is basically the standard acute and chronic toxicity, where what's going on, as you think, is a gross overwhelming of homeostatic processes, and these tend to be reversible processes, as long as you don't, you know, exceed your tipping point to do actual long-term damage.

These reversible processes usually include simple enzyme inhibition, receptor activation/inactivation, and you have an expectation in these cases of individual thresholds, although different people will differ in their thresholds in a population. So your variability is differences in the dose needed to cause an effect.

On the other hand, there's also quite a few cases where you have, at the heart of a causal process for adverse effects, irreversible processes; DNA mutation, that is, basically you have a DNA damage event that is not repaired by the time the DNA is copied next; destruction of alveolar receptor, destruction of most neurons.

If there are only a few irreversible events in the causal process, that is, mostly cancer, then I call these a molecular biological category. If there are many, many like in the neuron case or the FEV_1 or the chronic loss of lung function or blood pressure, I call these chronic cumulative because they accumulate over time with age and for normal processes and with some additional losses from whatever exposure you're dealing with. So, anyhow, what I want to suggest to you is that these different cases require different treatment for inter-individual variability.

So basically, for central acute overwhelming of homeostatic processes, the concept is that you have, you know, a fraction of people that are affected, who's basically -- basically where the dose has exceeded their individual limits for effect, and that's represented by this shaded area, and you can plot the Z scores of a series of dose-response points

versus the log of the dose or the dose, and you then get something like a plot like this.

This is a composite log probit plot of the ozone dose times severity of response relationship, where this upper line represents a 5% decline in FEV_1. FEV_1 is the amount of air that you can breathe out in 1 second following the ozone exposure. The blue line is 10% change, and the red line is 15% change. So you can see that they are all more or less parallel, and in this case as well, the slope of the line is an estimate of the reciprocal of the measure of variability, which is the -- it's basically 1 over the log GSD when you're doing a log plot, whereas the intercept is related in a fairly simple way to the median dose that's required to cause the effect.

Okay, variability and susceptibility for molecular and biological effects, that is, the cancers. Basically, the idea here is that dose-response relation at low doses is a composite of low-dose linear relationships. And I won't go into the linear justification here, but if you want, I will expand on that. So, basically, we have a probability of getting cancer by a specific age, according to a Poisson distribution, 1-kd where *k* is -- where *d* is the dose and k is lognormally distributed in the population. So we want to understand the

spread of that lognormal distribution.

At the population level, this implies a slightly convex dose response, an upward-pointed dose-response relationship between dose and fraction of people affected. The curve bends in this way as the most susceptible people are progressively eliminated or reduced as the dose is increased.

Chronic cumulative effects represent the accumulation of small, unreversed damage steps, like loss of specific neurons in Parkinson's disease or loss of structure of components in the lung leading to emphysema and the loss of lung function. Damage processes I'm going to skip over. And I'm going to skip over this.

This is some data that I analyzed sometime ago from the Six Cities study, and it's hard for you to see, I know, but this is the distribution of the FEV_1 levels in people with no smoking exposure at all or lifetime nonsmokers. And as you proceed to higher and higher levels of smoking -- this is 1 to 20 pack-years, 21 to 40 pack-years, 41 to 60 pack-years, etc. What you can see is that the mean shifts to the left in the direction of worse function and the distribution spreads out.

So I can model how much spreading would I expect, given expected variability in the smoking exposure in different dose

ranges, and how does that compare with what I actually observed in these data?

And basically, this is sort of a summary of applying this technique to two different populations, one, the Six Cities study and the smaller Tucson study. And basically what you see is that in a chi-squared analysis of the distribution of those observed FEV_1 levels, the p-values for no effect, no inter-individual variability are much less than one in a million, but that the two studies give me different optimal estimates of the likely inter-individual variability.

For the Six Cities study, we get something like, you know, a GSD standard deviation of about 1.9. For the Tucson study, I came up with an optimal value of 4.2, which is much larger. And I can put these two together in a Bayesian combination to get an overall probability against the function that's slightly more than 2.

So I think I want to -- because my time is just about up, I want to conclude with some conclusions. One is that mechanisms matter. Mechanisms producing the variability affect the forms of the distributions that are likely to be helpful. Mechanisms producing adverse effects determine how information relevant to variability should be analyzed and used in risk

assessments, but that meaningful quantification of variability and susceptibility for different kinds of effects is, in fact, possible.

(Applause.)

DR. DRESLER: Okay, our next speaker is Dr. Weihshu Chiu, and I would hope that I say that correctly.

UNIDENTIFIED SPEAKER: Weihsueh.

DR. DRESLER: Weihsueh, Weihsueh. Okay, thank you. And he is from Texas A&M University, and he will be speaking on Addressing Population Variability and Susceptibility in Risk Assessment, and he'll be online.

Dr. Chiu.

DR. CHIU: Hi. Can you hear me?

DR. DRESLER: Yes, perfectly well. Apparently, there's a bit of a time delay, maybe 10 seconds, for you to see the slides. But if you'll just say next slide, please, and that will be taken care of in here.

DR. CHIU: Sure, great.

DR. DRESLER: Thank you.

DR. CHIU: It's really a pleasure to be a part of this workshop, and I thank the organizers for the invitation. You know, I apologize for not being able to be there in person.

So you can go to the next slide.

So this is just my conflict of interest statement. I don't have any conflicts of interest related to the matter of this presentation.

And the next slide.

I'd like to begin with acknowledgements because this is really the work of many people together, and I'm pulling from many different collaborations and discussions and projects that I've been working on over the years, and I just want to make sure they're all acknowledged at the outset.

The next slide.

So I'm going to talk basically about two things. First is sort of an overview of the challenges in assessing population variability and susceptibility, and this is sort of more general, although it's definitely true both in generalized as well as for tobacco products in particular. And then I'll talk about basically current and emerging approaches for addressing some of these challenges, and dividing it up into talking about toxicokinetics, toxicodynamics, and the inter-dose response assessment. I'm not going to talk about variability in exposure. I think that was covered in other sessions.

So next slide.

So really existing risk assessments, you know, they talk about variability, and there's a lot of qualitative discussion. But in terms of quantifying, you know, quantitative evaluation, it's really quite limited, including the examples that I've shown here, you know, the cover pages for various articles about tobacco products.

So for cancer risk assessments, it basically assumes everyone has the same risk, except for early life exposures, and you know, maybe there are a couple of other examples, like radon and smoking, where there's some interaction with a co-exposure. But really, you know, the variability and susceptibility is not generally addressed.

For non-cancer assessments, the tenfold uncertainty factor still rules the day, and there are some examples of going beyond that, but it's still fairly rare. And especially if we're talking about all the different chemicals of concern in tobacco smoke, you know, there are very few of those. Is there any sort of deviation from the usual tenfold factor?

As I mentioned, exposure variability I'm not going to address here because it is actually more common to, and more standard of, the practice to address exposure variability, particularly because it's somewhat easier to measure than, you

know, the dose response and hazard aspect.

And these are all issues that are exacerbated in the case of tobacco products because of the multiple chemical exposures, and also importantly, because this is where we're not essentially deriving spaced doses of tobacco smoke. You know, in terms of the charge to the FDA, and this was mentioned earlier today, in terms of the regulatory paths, it's really about assessing incremental changes in exposure or similarity or dissimilarity.

So you're not starting from zero or trying to get as close to zero as possible; you're trying to see, you know, given the current state of things and whether a new product or a new situation has an incremental change. And most of the existing risk assessments really are built to that type of sort of marginal exposure, not margin of exposure but rather looking at marginal changes in exposure.

The next slide.

So really, you know, in terms of more on the data side, there's also a large number of challenges in trying to better evaluate variability and susceptibility. Now, there's a lot of this on slides, and you know, because I can't actually actively click slides, just had them all appear, but I'll just start at

the top.

For epidemiologic and clinical studies, first, the number of chemicals for which this is available is rather limited, and in general, there's enough difficulty, you know, in observational studies to even assess causality, let alone try to disentangle population variability and susceptibility.

In terms of animal bioassays, there are more data, you know, from experimental animals, across chemicals. It is limited by our understanding of interspecies differences, but you know, another important aspect to remember is that experimental animal studies are almost all homogeneous in terms of genetics, size, etc., all of these other factors that contribute to population variability in the human population.

And so as Linda Birnbaum once said, when you've done an animal experiment, you assess the hazard to, at most, one human.

So in terms of toxicokinetic models, again, there are relatively few that are actually available in terms of PBPK models, and there are also quite a limited number that actually analyze population variability or uncertainty for that matter, you know, as Dale mentioned.

For in vitro assays, this is sort of a new trend. In

going in vitro, we're going to be able to assess many more chemicals. Of course, there is still the issue of how we relate that to a health risk, but again, like the experimental animal studies, these are really genetically homogeneous in vitro systems, by and large. And so again, we're limited in our ability to assess variability and susceptibility based on these type of assays.

Adverse outcome pathways were mentioned earlier in the morning session. You know, they might have some promise, but they -- you know, there are a number of limitations. Particularly, that's for in the case of tobacco, where there are many different compounds as well as many different endpoints.

First is these AOPs are not really available for that many endpoints. A lot of the focus has been on things like skin sensitization and more shorter-term effects. They're currently very much qualitative and not quantitative. They're very linear in how they're constructed, whereas we know that these are multifactorial. And variability and susceptibility are not even on the radar screen really for most of these efforts.

And so all of these things then feed into, of course, the toxicity values and risk characterizations that we ultimately

use in risk assessment. So as a result, there really is no adequate addressing of variability and susceptibility beyond, say, using a tenfold factor. And then, of course, they don't -- many of them, particularly for non-cancer, as mentioned by George and others, they don't actually estimate an actual risk, which makes it difficult to make the sort of assessment of marginal changes.

Next slide.

So I'm going to focus really on these three topics here, toxicokinetics, toxicodynamics, and population-based dose-response assessment as sort of responsive to try to at least, you know, push the envelope in terms of moving things forward. I'm going to begin with toxicokinetics, and the focus here is really on animal bioassays, animal experiments, as well as toxicokinetic models.

So the next slide.

So in terms of population variability and toxicokinetics, I'm just showing here, you know, the usual RfD kind of approach, and they're all divided by uncertainty factors and the human variability factors on the right side divided into a 3 and 3 for toxicokinetics and toxicodynamics.

So right now we're talking about the toxicokinetics, and

really PBPK models are, you know, kind of stated as they are here. One of the earlier speakers talked about dosimetry, and so I'm not going to dwell on this very much, except to talk about some of the new type of tools and data that might be available.

So population PBPK modeling is just basically taking a PBPK model, what you see in the middle, and doing a Monte Carlo simulation on it. So all the different parameters in the model have distributions that reflect the variability in the population, and those are just run through the model many, many times, and then you end up with, on the right, a probability distribution of the internal dose metrics that you're interested in, whether it's a target tissue or a blood concentration or some metabolite. It's basically you put a distribution in, then you get a distribution out.

And most of the approaches -- and this is the call-out at the very top left. In most cases, these are fixed distributions in most of the published applications. They're sort of, you know, basically axiomatic or postulated distributions as to what these -- how these parameters are distributed in the population, you know, based on -- in many cases for physiological parameters at least, on population-

based data like NHANES or cadaver data.

On the other hand, Bayesian approaches add the component of uncertainty in that we're not actually sure how variable some of these parameters are, particularly when we get the chemical-specific parameters. You know, these Bayesian approaches then are able to address the uncertainty we have in how much variability there is.

Next slide.

So fortunately a lot of the variability, you know, as a result of PBPK models is due to physiology, and we have a fairly good handle on that kind of variability. And, in addition, it has the benefit of being the same across chemicals, as long as the same model and the same parameters are being used.

And also we have some good idea as to the range of possible toxicokinetic variability because, particularly for drugs, there is actually clinical population PK data. And Dale has been instrumental, over the decades, in collecting and compiling these types of data. And just the distribution shown here is for the ratio between the median and the 1% most sensitive individuals, and that has a range of a little more than 1, so about almost a factor of 10 across chemicals that

he's looked at. This is for AUCs, I think, with an essential value of around 2½.

So, you know, chemical-specific data, of course, can narrow the range of where we think the actual distribution of variability is. But unfortunately, outside of drugs and a few other high-profile chemicals like benzene or trichloroethylene, there really isn't a lot of human population kinetic data on these individual chemicals.

So one question then: Is there some sort of experimental model that can mimic human variability, and how well might that work?

Next slide. So hopefully we're on Slide 11 now.

So one idea is to use, actually, strains of strain variability in a rodent model to try to assess toxicokinetic variability. So this is an experiment which has 17 different strains of mice, looking at trichloroethylene, and there are different internal dose metrics, and these are metabolites, TCA, DCA, DCVG, and DCVC. As you can see just from these three sort of strains, including the B6C3F1 strain, you know, there is substantial variability, particularly in the bottom two, the GSH conjugation metabolite.

And so this was then analyzed using a population PBPK

model, and that's why in the cursor you see these error bars, because that accounts for both the uncertainties between strains -- I mean the variabilities between strains where the uncertainty even within a strain, you have uncertainty as to the actual concentration and response curve.

So click to the next slide.

So on the right, here, this is the ratio -- on the top right is the ratio of the oxidation of the GSH conjugation across strains. So each of these estimates has an error bar because, using a Bayesian approach, we are accounting for both uncertainty and variability. But as you can see, there is a fair amount of uncertainty for each individual strain, but you know, there's a definite contribution from variability as well, between strains.

And the bottom is TCA produced, which is a mouse beta metabolite, and as you can see from these graphs, you know, the scales are very different. So there's definitely more variability with respect to the oxidation versus GSH conjugation balance than there is for TCA alone.

And so the table in the middle, on the right column is the intra-strain variability. This is a ratio between 95th and 50th percentile for intra-strain variability for three

different dose metrics: TCE oxidized, TCA produced, and then conjugation of TCE with GSH. And as you can see, you know, as you go from top to bottom, there is more variability depending on the particular dose metric you're interested in.

So, you know, one message in general for these population PBPK models is, you know, how much variability there is will often depend on what particular internal dose metric you're concerned about, and so that's where the mechanistic data in terms of the active moiety becomes very important.

Now, the left column is actually based on human data, individual human data from basically pooling studies from, you know, a span of maybe 40 or 50 years or so, and as you can see, the mouse variability estimates from multiple strains and the human population variability estimates from controlled human exposure studies are actually very consistent. You know, the error bars completely overlap. And so this suggests, at least as proof of principle, that it might be possible to use an experimental rodent strain in order to try to use it as a surrogate for human variability.

The next slide.

So our next step. So that was multiple inbred strains. So the next step is to have some sort of reproducible genetic

diversity that's sort of a little bit more heterogeneous than just the individual 17 strains. And this is where there has been recent mouse genetics that have been working on what's called a collaborative cross, which is basically taking eight founder strains, including some outbred strains, and then crossing them for many generations until you -- and then developing inbred strains from those crosses. So now there are several hundred collaborative cross inbred strains, each of which is a mosaic of the original eight founder strains.

And on the right are some preliminary data from experiments, again using trichloroethylene, and you know, across the x-axis is the different strains, and then the top is CYP2E1 in the liver, the expression level. And as you can see, there is a very large amount of variability, you know, much more than tenfold across those 50 strains or so. And then on the bottom is the TCA concentrations in the liver, again, you know, a very large range of variability.

But it's interesting that, you know, if presumed -- it's been presumed that CYP2E1 is the predominant enzyme for metabolizing trichloroethylene. And yet two strains with very low relative levels of CYP2E1, and with very similar low levels, have completely different values for the

trichloroacetic acid in the liver, which just goes to show that toxicokinetics is really a complex trait and that, you know, in addition to this sort of bottom-up kind of looking at one enzyme at a time or one parameter at a time, there is going to be a need for kind of empirical data from top down in order to really properly estimate any types of population variability.

So next I'm going to talk about toxicodynamics, and here it is to focus on sort of the in vitro assays and whether we can bring -- you know, we can add some genetic variability and heterogeneity to how we test in vitro. The next slide. Hopefully we're on Slide 15 now.

So population variability in toxicodynamics. So this is the other three of the uncertainty factors for human variability of 10. And again, based on vanguarding work from Dale, we have some idea as to the range of possible toxicodynamic variability, again, mostly from drugs. And here it's a little bit more variable, you know, going up to a factor of 29 and with a median of about 3, a little more than 3. But there are virtually no examples of using chemical-specific estimates of toxicodynamic variability in risk assessment.

So the next slide.

So one idea, which was published last year in a subsequent

publication that's been submitted this year, is to basically go in vitro. And here we're talking about human data. So what we did in the study is we took basically lymphoblastoid cells from the 1000 Genomes Project, so this is a project that looks across -- has people from across the globe, from different ethnic origins and geographic backgrounds. So we have a sample of about 1,000 individuals from which we have lymphoblastoid cells that we did high-throughput in vitro screening for cytotoxicity.

And then on the other dimension in terms of chemicals, we took 170 compounds that are structurally diverse and tested all of these in vitro, so this 1,000 individuals by 170 compounds, each of which was done in concentration response with replicate and -- you know, badge replicates and stuff like that. So this was a huge study. It was assisted by, you know, the facilities at NCAD, and the idea here was to look at, well, what can we -- first, you know, can we actually see variability across individuals, and then are there differences across chemicals and how much variability there is.

And just as examples on the bottom, I took four compounds that might be potentially relevant to a tobacco product -- they're from, you know, one of the lists that FDA published --

just to illustrate that, you know, on the left is cadmium chloride, which has about a twofold difference across individuals in terms of the concentration leading to the beginnings of cytotoxicity, catechol at about threefold, and then over eightfold for some of the mercury compounds.

So, you know, again, a proof of principles that definitely you can see population variability, and this was reproducible because we had the replicates, and we knew that this wasn't noise. And these estimates, by the way, have been -- have measurement error already subtracted out or accounted for. So there's both population variability, there's chemical-to-chemical variability, and there's chemical variability and how much population variability there is.

So what are some, you know, possible next steps in this next slide? So that was lymphoblastoid cells and cytotoxicity. So really we want to kind of have really a diverse set of phenotypes as well as cell types to test in vitro. So induced pluripotent stem cells offer a promise to be able to do this with multiple cell types eventually from multiple individuals.

And then we can, for each cell type, measure cell type-specific measures of function or toxicity, you know, shown on the right here. Each types of cell will have -- you know,

there's general kind of toxicity, but there's also cell type-specific functions that we want to be able to measure to see whether those functions are being disrupted by chemical subgroups.

So I'm sure this sounds like kind of an aside, but there actually is one example thus far -- next slide -- in which we're looking at cardiotoxicity, and this is a grant from EPA to look at population toxicodynamic variability using cardiomyocytes. So these are from induced pluripotent stem cells that have been differentiated into cardiac cells, and again looking at a diverse set of chemicals, 140 chemicals, and I've listed some of the ones that are on one of the HPHC lists for tobacco products here.

But this is just to show the box plot in the middle, this is the peak frequency for these cardiomyocytes. So these cells, they actually beat in vitro, and you can detect their beating patterns. Those are the traces on the right. And so there is definitely variability just in baseline, which is what the box plot is, in terms of beats per minute.

And as you can see in the first set of graphs for untreated DMSO-treated cells, the top one is a fast beater, and the bottom one is a slower beater. And then treating with

sotalol, which can cause QT prolongation, which is a major concern for drugs but also potentially for environmental chemicals and tobacco smoke as well, tobacco products as well, you can see that there is also variability in the phenotypic outcomes across these individuals, for the same dose, for the same concentration of the same chemical. You know, again, it's a proof of principle that we can potentially assess more complex phenotypes in a differentiated cell type across individuals and across chemicals.

The next slide.

So the final topic which I'm going to close with is talking about probabilistic dose-response assessments, and I'm sure there's going to be more discussion of this tomorrow, so I'm not going to dwell on this too much.

But I really wanted -- if you go to the next slide after this -- to revisit what we mean when we talk about these uncertainty factors. So, you know, because the animations are all done, you already know the answers here. But the question is really what do we mean when we apply sort of a default factor of 10?

So in some forums, I've seen them talking about the least sensitive to the most sensitive humans being tenfold, and

that's definitely not what the tenfold factor means. You know, first of all, it's from the least to the most rather than from a typical to a more sensitive. And also the range will just depend on sample size. The bigger your sample size, the wider the range typically becomes.

The second one is also not true, that sensitive humans are always 10 times more sensitive than typical humans, because the default factor is meant to be conservative, and it's meant to be protective as well. So what it really is intended to mean is that for most chemical endpoints, sensitive humans are no more than 10 times more sensitive than typical humans. I'm not plugging it into a TK and TD factor of 3 and 3. It's really intended to cover "most" humans or "sensitive" humans.

So the next slide.

So these ideas of "most" and "sensitive," though, of course, are not typically specified, you know, particularly for a generic assessment and for the reference dose that results from it. So the idea is just simply that if you have a distribution and the 10 is somewhere over to the right tail, and the value of 1.1 is somewhere over on the left tail -- but again, we actually have historical data, you know, that Dale has collected, as to what that distribution might actually be.

And so this distribution here shows combining the toxicokinetic and toxicodynamic variability, and this is, again, distribution across chemicals.

So the particular values here are for 5% being defined as the more sensitive, and therefore, you know, based on that definition of sensitive, the default factor of 10 corresponds to about a 90th percentile in this distribution. But, you know, who's to say that 5% or 90th percentile is the right value, can depend on your particular decision context. In some cases, you might want a central value. In some cases you might want to go further out, you know; you want to have high confidence, 95 or 98% confidence. And then in terms of how sensitive an individual you want to protect, that's more of a risk management level of protection decision as well.

So just as an example, if you click, now this will now change to 1%, defining sensitive as 1%, and you know, that changes what your distribution is, and it goes from 2 to 42 in those confidence bounds on the distribution. And then the factor of 10 is now only a 70th percentile; it only covers 70% of chemicals if you want to protect out to the 1%.

Next slide.

So similar issues, of course, apply to each component of

the RfD and RfD itself. I'm not going to go through these slides just in the interest of time, because most of the issues are covered in a couple slides later. But just the issue of, you know, what does conservative mean? How much are you protecting? How conservative? You know, those aren't really quantified in terms of these factors or the RfD.

The next slide.

So in work that Dale and I have been involved in with the WHO, the idea is to go potentially beyond RfD to what we call the target human dose, the HD_M^I. So that is defined as the dose at which a certain fraction of the population shows a magnitude of effect, a particular magnitude *M* or greater.

And this is illustrated maybe more easily on the right with the dose-response curves, so the idea being that each person has their own personal dose-response curve and as the -- you know, that's the left one, but 1% most sensitive individuals will have effects at a lower dose, and then there is a confidence interval on that.

So you could specify the point of departure as being, or the reference dose even being -- essentially protecting up to the most sensitive 1% of the population or, you know, a 10% change in some liver enzyme or body weight, liver weight/body

weight ratio or something like that, whatever your metric is, so thereby making explicit sort of how much of the population you're intending on protecting and at what level of confidence.

Next slide, please.

If calculated very similarly to how an RfD is calculated, you just essentially replace all of these factors, uncertainty factors, with distribution. But now the distributions actually have additional indices attached to them. So, for instance, instead of a NOAEL, you have benchmark dose with this particular magnitude of effect *M*.

And then for the uncertainty factor for human variability as opposed to just a single factor, that has another index *I* attached to it, which is related to how much residual incidence in the populations you're willing to affect, so 1%, 5%, etc. And then the end result, then, is a distribution as well, which then addresses the uncertainty in all of these calculations.

And then you can -- if you want, then, sort of a conservative estimate, you could use the lower bound, lower confidence bound on this uncertainty distribution defined as sort of a probabilistic version of the RfD.

The idea, if you go to the next slide, then, is that the target human dose is basically a more precise definition of the

RfD. And so on the left of this slide I have, you know, the typical definition of RfD, and on the right there are various words in the RfD definition that are made quantitatively precise in terms of what they're referring to.

So the idea of "likely" is then talking about statistical lower confidence bounds with a specific percent confidence, you know, the idea of protecting the human population, including sensitive groups. Then you're being explicit as to the fraction of the population that you're protecting *I*. And then without the idea of the deleterious effects, we can now quantify that as well, using the benchmark response levels or magnitude or severity of effect.

So, in essence, we should be turning things around and saying all of these RfDs that we've been calculating are really approximations of an HD_M^I and should thus be viewed as not anchoring to the older RfDs, but we should kind of recalibrate all our old RfDs based on this sort of more quantitative approach.

Next slide.

So for applications, this can, of course, replace the RfDs and RfCs, while also being explicit as to the confidence levels and population variability as well as the severity of the

effects that we're talking about. And it explicitly addresses population variability, and it provides the dose-response function through this magnitude of response *M*, and therefore it can be applied in the situations such as is common for tobacco products where we're talking about incremental changes in dose or in constituents of the dose.

And theoretically, it could also be applied for cumulative risk because now we can look at all the different compounds and how they affect a particular endpoint and accumulate those together rather than the current RfDs are really -- you know, they're each based on probably a different endpoint, and so it's very difficult to accumulate those in any sort of quantitative way.

And then, also, we can incorporate chemical-specific data on toxicokinetic and toxicodynamic variability, basically using the approaches that we've been through in the first two parts of the talk, and then, of course, characterize uncertainty.

Next slide.

This is also applicable to cancer dose response, so bringing the population -- as I mentioned at the very beginning, cancer dose response really doesn't address variability at all, not even with a tenfold factor or anything.

But we can apply the same approach to cancer dose responses as well. And I'm referring here to a presentation that I did as part of an FDA symposium last year in which I talked about this a little bit more.

Next slide.

So just to summarize, and I realize being on the phone that I don't know when my time is up, so hopefully I didn't go over too much, but to conclude, there are many different challenges in addressing population variability and susceptibility in risk assessment.

There are many established approaches for toxicokinetics and there's -- you know, the best practice is really Bayesian population PBPK modeling, but it's also limited by the availability of human toxicokinetic data. But there's emerging population mouth data that could be used to help inform that.

In terms of toxicodynamic variability, this is an emerging area, really, and there is some proof of principle using in vitro data. And hopefully in the future, iPCS-based human population data can be used to better get a handle on how much toxicodynamic variability there is for a particular compound or mixture.

And then, finally, these are really not that useful in and

of themselves and are best utilized if we can incorporate them into an overall probabilistic dose-response assessment approach.

And I think, you know, based on my work with Dale and with the WHO, we think that this is really ready for implementation, and it can better incorporate what we know from historical data as well as chemical-specific data on toxicokinetic and toxicodynamic variability and provide dose-response characterizations that, I think, would probably be more -- have more utility in the tobacco product risk assessment area than the current usual historical approaches.

And that's my last slide. Thank you very much.

DR. DRESLER: Well, you all, it has been a long day, but you know, questions. So maybe if we just take maybe 5, 10 minutes since we've hung in there so long, to be fair to our last two speakers. Do we have questions?

Dr. Chiu, what approaches may be used to extrapolate from the concentrations applied to cells into a human-relevant dose? So what approaches may be used to extrapolate from the concentrations applied to cells into a human-relevant dose?

DR. CHIU: Right. So this is where this concept of in vitro to an in vivo extrapolation or a reverse toxicokinetic

comes in. So, again, using PBPK models, sometimes -- you know, in some cases, highly simplified models like a three-compartment model, but that can be used to basically back-calculate what the dose would be to get sort of a serum concentration that would be equivalent to what the cells in culture were exposed to. So that's assuming that the cells in culture would be exposed in vivo to something similar to, like, a blood concentration.

DR. HATTIS: Yeah, I'm afraid I have a bit of a sad story to tell in this regard. I tried to use, to compare in vitro, IC50s with in vivo LD50s, the most simple comparison to make, and I found that the IC50s, the in vitro level, predicted about 1% of the variance of the logs of the LD50s. And the reason which was, I thought, not good enough, the reason why the comparison failed was in part because of the large number of anti-cholinesterase agents that were in the groups that I had both IC50s and LD50s, and that it makes sense because the IC50s are solely based on single-cell assays of toxicity or at least gene induction, whereas, you know, many of the actual measures of mechanisms of toxicity involve multiple cells like the anti-cholinesterase action where you need inhibition of the changes in the transmission of signal between adjacent neurons.

So I think that it's an interesting idea to try to use these in vivo -- in vitro, too. So I think you have to have at least some major -- some representation of major cell-cell communication types of toxicity before you can hope that it will be successful.

DR. DRESLER: Okay. And then I have two related questions, one that would be for each of you and your topics. But Dr. Hattis, you illustrated techniques that describe variability of distributions for chemicals with sufficient data. What approaches may be used for chemicals where data are lacking?

DR. HATTIS: Well, basically what you need to do is to basically -- basically any uncertainty can be characterized by taking a bunch of cases that are similar to the ones that you have and analogizing or studying them in such a way that you can say how likely is it that I'd be wrong by a particular amount if I adopted a straightforward translation? So this is basically the route for replacing the uncertainty factors. But the same thing can be done the other way, but it takes a bit of work, and you basically assemble a database where the source information and the recipient information are known, and you say my uncertainty is represented by the distribution of

translation factors that would be needed to convert one source of data to the other.

DR. DRESLER: Okay. Dr. Chiu, something similar. You described approaches to use data to adequately describe population susceptibility and variability. So what approaches would you use for chemicals where data again is lacking?

DR. CHIU: Okay, can you hear me?

DR. DRESLER: Yes, we can hear you.

DR. CHIU: Okay. All right, I was just making sure I'm still there. So I think in the absence of chemical-specific data, there is -- you know, the crudest approach would just be to take what we know from chemicals we do know something about, but use that distribution essentially as -- you know, basically, we're not sure what the value is and how much variability there is, so we're just going to assume our chemical might be a random draw from the chemicals we do know something about and then account for that uncertainty in the variability.

Now, it is possible maybe to use QSAR or some other, you know, computational approach to try to get a better estimate, you know, try to narrow that uncertainty. Maybe we can have a structurally similar compound or, you know, basically an analog

based on chemical structure or property data. So that could be another approach to try to -- you know, in the absence of data, to try estimate what those values might be.

DR. DRESLER: Okay. And one last question. Are there any available risk frameworks for integrating environmental factors, such as non-chemical stressors, that contribute to the disparities in health outcomes associated with tobacco products? So any available risk frameworks.

DR. HATTIS: I guess I would have to go back to, you know, the semblance of information we do have about drugs and selected environmental chemicals. But those are not -- those are far from perfect because you don't have -- in the chemicals, for example, of drugs that are tested in Phase I for kinetics, it would be madness for a drug company to include a fully representative sample of the population in the tested, you know, because you would not want to have your chemical tainted with a possible adverse reaction from somebody who was really pathological in five different dimensions.

And so the normal people included in Phase I drug studies cannot possibly be fully representative of the diversity of poor and rich and old and young people with different kinds of maladies, and you need to have some other route to really take

those sources of variability into account.

As a general matter, I want to note that this was all started, this uncertainty factor tradition was started with FDA researchers Lehman and Fitzhugh in 1954, and the hundredth anniversary of the Lehman and Fitzhugh paper is rapidly approaching in 2054, and I hope that as a society we set a goal that by 2054, we're no longer dividing by the number of fingers on our hands.

(Laughter.)

DR. CHIU: Can I just add? In terms of risk parameters, I think one possible area in which this framework could -- I mean, there are some examples with some continuous endpoints like IQ and maybe some cardiovascular endpoints like blood pressure for which we can assess kind of what the impact of the chemicals are on that particular biomarker. But then that biomarker, in terms of ultimate risk for some, you know, cardiac effect or in terms of the larger societal effects from IQ decrement, you know, can then take into account the population variability and those baseline characteristics.

So, for instance, for blood pressure, you know, if we're increasing everyone's blood pressure by a small amount, that small amount of increase will have a differential effect across

the population. For some people that's a larger increase in their cardiovascular risk than for others.

And so incorporating kind of a biomarker approach in which we have -- and then having models in which we can quantify the effects of multiple risk factors, not all of which might be affected by chemicals but which have significant variability on baseline in the population, might be a viable approach.

DR. DRESLER: Okay, thank you. Thank you both to Dr. Chiu and to Dr. Hattis for closing out this day and for their presentations and hanging in there with us. And thank you very much to the audience also, that we have gone over a bit, and it's been a long day with a lot of information. So kudos to you all for hanging in there.

Tomorrow morning we start at 8:30, okay? So we'll start tomorrow at 8:30. See you then.

Thank you.

(Applause.)

(Whereupon, at 5:19 p.m., the meeting was continued, to resume the next day, Wednesday, November 16, 2016, at 8:30 a.m.)

C E R T I F I C A T E

This is to certify that the attached proceedings in the matter of:

RISK ASSESSMENT OF TOBACCO PRODUCTS: A PUBLIC WORKSHOP

November 15, 2016

Hyattsville, Maryland

were held as herein appears, and that this is the original transcription thereof for the files of the Food and Drug Administration, Center for Tobacco Products.

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ED SCHWEITZER

Official Reporter

Professional Video Associates, Inc.
2515 Saint George Way
Brookeville, MD 20833
301-924-1556