MEETING

STATE OF CALIFORNIA

ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

PROPOSITION 65

CARCINOGEN IDENTIFICATION COMMITTEE

ZOOM PLATFORM

CALEPA HEADQUARTERS BUILDING

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COASTAL HEARING ROOM

SACRAMENTO, CALIFORNIA

TUESDAY, FEBRUARY 27, 2024

10:00 A.M.

JAMES F. PETERS, CSR
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APPEARANCES

COMMITTEE MEMBERS:

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Ahmad Besaratinia, PhD, MPH

Jason Bush, PhD

Catherine Crespi, PhD, MS

David A. Eastmond, PhD

Joseph Landolph, PhD

Thomas McDonald, PhD, MPH

Mariana Stern, PhD

Sophia Wang, PhD

STAFF:

Lauren Zeise, PhD, Director

Kristi Morioka, Senior Attorney

Martha Sandy, PhD, MPH, Chief, Reproductive and Cancer Hazard Assessment Branch

Meng Sun, PhD, MS, Chief, Cancer Toxicology and Epidemiology Section, Reproductive and Cancer Hazard Assessment Branch

Kiana Vaghefi, Proposition 65 Implementation Program

APPEARANCES CONTINUED

SPEAKERS:

Gary Ginsberg, PhD, Director, Center for Environmental Health, New York State Department of Health, Yale University School of Public Health

F. Peter Guengerich, PhD, Vanderbilt University

Vasilis Vasiliou, PhD, Yale School of Public Health

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PROCEEDINGS

DIRECTOR ZEISE: So I'd like to welcome everyone to this 2024 meeting of the Carcinogen Identification Committee so nice too see all of you in person. We also have participation online by the public as well as in the room. So nice to see you all. My name is Lauren Zeise. I'm Director of the Office of Environmental Health Hazard Assessment. That's a department within the California Environmental Protection Agency. It's a lead agency for assessing health risk posed by environmental contaminants in the state of California.

So as we get started, we're going to just have a few housekeeping items. First in an event of an emergency, the emergency exits are the double doors directly behind you for those of you in the room. And in the front of the room to the left is a -- also a lighted exit. And so you can access the restrooms by going out the back double doors turning to the left and walking to the end of the hall.

So today, we have our main agenda. We have two important agenda items plus an update on -- of Proposition 65 activities. So the first item we're going to be looking at enzyme polymorphisms and susceptibility for carcinogenicity. And we're delighted to have such distinguished speakers to help us with this item and think

about it in terms of our hazard identification documents and decisions that we make under Proposition 65 as well as a variety of other -- of our OEHHA program activities. So really delighted to have that item covered.

And then the second item is an opportunity for the Committee to provide us input on a proposal to streamline several sections of our cancer hazard identification documents. And these are the documents that are provided to the Committee to inform their decisions about whether or not a chemical should be listed under Proposition 65 as a carcinogen. So we're really looking forward to the Committee's discussion and input on this item.

And for the third and final agenda item, staff are going to provide updates on various Proposition 65 regulatory and other activities.

So today, there are not going to be any Proposition 65 listing decisions. So, that is what we have in front us today and we'll be taking a 45-minute break for lunch around noon and a short 15-minute break sometime in the afternoon.

The meeting is being recorded and transcribed, and the transcription will be posted on OEHHA's website.

So another kind of housekeeping item is regarding public comment. So during the meeting, there will be

opportunity for the public comment on the enzyme polymorphisms and susceptibility to carcinogenicity item. And so I think slides are being shared, yes.

Okay. So individuals who are in person and wish to make an oral comment are asked to fill out a blue comment card, and that's at the back of the room. And then when called on by the Chair, you would approach the microphone and state your name, affiliation, and provide your comment.

And then for those of us who are attending virtually and would like to make a comment, they're asked to join the Zoom webinar. And for information on how to join Zoom, that's shown on the slide. And you can see, you go to the HTTPS site noted on the slide to register for Zoom. HTTPS://bit.ly/registercic2024.

So you'll receive a link to join the webinar at the end of the registration process. And if you -- and if you provided a working email address, you'll also receive an email with a link to join the webinar.

And many of you may be joining via CalEPA webcast. So you'll be able to watch the meeting if you're joining that way, but you won't be able to make a comment. You'll have to join the Zoom to speak.

Okay. So when requested by the Chair, individuals on Zoom webinar may queue to provide oral

comment by using the raise hand function. And then when your name is called, you'll be Provided the opportunity for public comment. You'll be prompted to unmute yourself. You'll unmute, state your name and affiliation and provide your comment.

And if you like to present slides during your public comment and have not already sent them, please email them now to P65public.comments@oehha.ca.gov. And that's also on this slide. Okay. So public comments will be limited to five minutes per commenter.

All right. Now, I'll turn to introducing the members of the Carcinogen Identification meeting[sic]. So I'm pleased to see you all, and we'll introduce you. As I introduce you, please state your name, affiliation, and position.

So Dr. Besaratinia.

COMMITTEE MEMBER BESARATINIA: Good morning, everybody. My name is Ahmad Besaratinia. I'm a professor at the Department of Population and Public Health Sciences at University of Southern California in Los Angeles. Thank you.

DIRECTOR ZEISE: Great. Thank you.

Dr. Bush.

COMMITTEE MEMBER BUSH: Good morning, everyone. Jason Bush, professor of cancer biology and Associate Dean

for the College of Science and Math, California State University, Fresno.

DIRECTOR ZEISE: Dr. Crespi.

COMMITTEE MEMBER CRESPI: Thank you.

Can you hear me?

Yeah.

Cate Krespi. I'm a professor of biostatistics at the UCLA Fielding School of Public Health.

DIRECTOR ZEISE: Dr. Eastmond.

COMMITTEE MEMBER EASTMOND: Dave Eastmond. I'm a Professor Emeritus, University of California, Riverside.

DIRECTOR ZEISE: Dr. Loomis.

CHAIR LOOMIS: Thank you. Dana Loomis. Recently retired from the Plumas County Public Health Agency and the Desert Research Institute in Reno.

DIRECTOR ZEISE: Dr. Loomis will be serving as Acting Chair today.

Dr. Landolph.

COMMITTEE MEMBER LANDOLPH: Joe Landolph. I'm associate professor of molecular microbiology and immunology and pathology at the Keck School of Medicine. I'm also a member of the cancer center there in Los Angeles, California

DIRECTOR ZEISE: Thank you. Dr. McDonald.

COMMITTEE MEMBER McDONALD: Tom McDonald, head of

product safety at the Clorox Company in Pleasanton, California.

DIRECTOR ZEISE: Dr. Stern.

COMMITTEE MEMBER STERN: Good morning, everyone. I'm Mariana Stern. I'm a professor of population and public health sciences at the Keck School of Medicine of USC In Los Angeles and the Associate Director of Population Science at the USC Norris Comprehensive Cancer Center.

DIRECTOR ZEISE: And Dr. Wang.

COMMITTEE MEMBER WANG: Hi. I'm Sophia Wang. I'm a professor at the City of Hope Comprehensive Center and Beckman Research Institute in the Division of Health Analytics.

DIRECTOR ZEISE: Okay. So welcome, Committee, and we really appreciate the time you're taking to come to this meeting, provide us advice at the meetings. Thank you so much.

Okay. Now, I would like to introduce OEHHA staff. And so I invite you to raise your hand as we -- and maybe even stand as we walk through and introduce you. So first, Dr. Elaine Khan, Chief of OEHHA's Pesticide and Environmental Toxicology Branch and Acting Deputy Director for Scientific Programs.

All right. And then for the Reproductive and

Cancer Hazard Assessment Branch: Dr. Martha Sandy, the Branch Chief; Dr. Meng Sun, Section Chief of the Cancer and Toxicology and Epidemiology Section. And then staff of the Cancer Toxicology and Epidemiology Section that are joining us today, so Drs. Feng Tsai, Gwendolyn Osborne, Karin Ricker, Kate Li, Neela Guha, Sarah Elmore, and Vanessa Cheng. So nice to see you all.

All right. We'll now turn to the Office of External and Legislative Affairs, Proposition 65 Implementation Program. So Dr. Amy Gilson, Deputy Director for External and Legislative Affairs; Tina Cox, Senior Environmental Scientist, Chief -- Section Chief of the Proposition 65 Implementation Program - and this is Tina's first meeting, welcome - Kiana Vaghefi, Environmental Scientist, Proposition 65 Implementation Program, Ester Barajas-Ochoa, Analyst, Proposition 65 implementation program.

And then from OEHHA's Legal staff, Kristi Morioka. So welcome all.

All right. So let's see. Now, we're going to have our legal representative here Kristi. She's available for the whole meeting. Thank you for coming in person, Kristi. And so if you have -- feel free to ask Kristi any clarifying questions or any OEHHA staff clarifying questions during the meeting. If they don't

have the answer, they'll do their best to find it and report back to you all.

All right. So also as a reminder that the Bagley-Keene Open Meeting Act applies to this meeting. Kristi, since you're in person, do you want to say a little more or...

SENIOR ATTORNEY MORIOKA: Let me see. Does this work? Okay. So just for the Committee members, remember that all topic discussions and deliberations need to be conducted during the actual meeting, not on brakes, not on lunch, or with individual members of the Committee whether you're in-person, on or offline, phone, email chats or text messages.

Thank you.

DIRECTOR ZEISE: Thanks, Kristi. All right. Now, I'll be turning the meeting over to Dr. Loomis the Acting Chair for today.

CHAIR LOOMIS: Thank you, Lauren. Good morning, everyone. I want to particularly thank Committee members who've traveled here for this meeting today, it's certainly going to be a novel format for me and all the rest of us. I gather there hasn't been an in-person meeting in 5 years. So I'm going to have to learn how to navigate this in-person. You know, I can't see the whole committee at once, for example. So I'll do my best to

call on the people when they want to speak. But if not, you may need to get my attention.

Thanks too to everyone from the staff and the public who's here this morning. I drove in early today and -- from the high Sierra where the temperature was 23 degrees and the snow was all around the highway to 53 degrees at Auburn. I just marvel at what an amazing state we live in here. So thanks everybody for being here.

Now, we'll move on to the first agenda item on enzyme polymorphisms and cancer susceptibility. So, we have a couple of invited speakers. I will introduce the first one. This is Dr. Gary Ginsberg. He's Director of New York State Department of Health Center for Environmental Health, also a professor at Yale University School of Public Health. He's worked collaboratively with the U.S. EPA and various academic researchers for many years on a range of projects that have focused on life stage and genetic polymorphism-based susceptibility factors. He's served on a number of federal scientific review panels as well as National Academy of Sciences panels. So Dr. Ginsberg will take the floor and it's all yours.

(Thereupon a slide presentation).

DR. GINSBERG: Thank you, Chair Loomis and I appreciate the invitation to come out and meet again some

old colleagues, not old in age, but just --

(Laughter)

DR. GINSBERG: -- long-standing here on the west coast, as well as it's always a pleasure to talk about interindividual variability and susceptibility factors. So I'll focus on some of the research that I was involved with that came about through a cooperative agreement between U.S. EPA and the State Of Connecticut where I was the State toxicologist for a long time. Looking at these individual variances, we realized that the molecular epidemiology literature was exploding with information about what we call single nucleotide polymorphisms, which are essentially just mutations in various genes that affect either the protein function or the inducibility of the protein. And so as this epidemiology evidence was building for their effect, their phenotypic effect on vulnerability, we realize that there wasn't really a good cataloguing of the various SNPs, the various mutations and key genes, and key functional pathways.

So we wanted to create a database that would catalog all of this and then talk about risk implications using some Monte Carlo methods. So I'm going to talk about that, but then also focus on couple of examples that show just how influential some of these SNPs can be in terms of -- especially when you start compiling the

various ones in the same individual. What does it look like if you have not just one polymorphism, but if you have 3, 4, or 5 polymorphisms within the same individual. So let's try this. That didn't work.

No, let's try that. Still not working.

Do I have to point it somewhere else?

Oh, it just moved.

Okay. Let's try it again. Okay, so now if we go backward. All right, now it's moving forward. I'm sorry. Okay. It's just -- there's a delay. I guess I have to be more patient.

(Laughter).

[SLIDE CHANGE]

DR. GINSBERG: Okay. So just to do some level setting. The early evidence of genetic polymorphisms and their influence on phenotype. There was in the 1950s clinicians recognized that the antitubercular drug isoniazid was creating side effects along the lines of neurotoxicity in about 4 to 17 percent of the subjects receiving the drug. And it was then understood that there was metabolism, urinary -- as evidenced by urinary metabolites of isoniazid that were clearly different in those who were more susceptible to the side effect and that was termed the slow acetylator, n-acetyltransferase, or NAT as we affectionately refer to it as, the acetylator

phenotype associated with that side effect.

And then around the same time, maybe a bit later, there was an antidepressant nortriptyline, that was found to vary widely in the population in terms of its pharmacokinetics, 40-fold variation, and then the -- it was identified as a cytochrome P450, or CYP2D6 variation, poor metabolizer, or a rapid metabolizer, or extensive metabolizer started segregating out. And with those examples, before -- well before the genetics were understood, probed substrates started being used. So for example, for CYP2D6, debrisoquine, a muscle relaxant that is pretty innocuous was used to phenotype populations and understand how these genes are inherited. And for a couple at least, it was early recognized autosomal recessants -- recessive inheritance. And so there's now a wide variety of probe drug substrates that can be used to understand this kind of variability for some of these pathways.

And then with the advent of more advanced genotyping methods, these genes were shown to have many variable locations, or many SNPs. And some of them, of course, being in the reading frame and being directly affecting protein function, others being upstream in the promoter sequence and affecting the inducibility of the gene or the expression of the gene. And others could be

completely silent.

And then -- so with that evidence, increasingly then there were studies that were done either in cell culture systems or in populations molecular epidemiology -- you know, captioning signal on top of the slide -- but the influence of SNP -- these SNPs on phenotype became a major focus on a mechanistic basis, you know, how is these -- how does the protein change affect the way the protein works in terms of its enzymatic function.

Then population studies increasingly demonstrated out allele frequencies very important, you know, how much of the population is affected by these kinds of influential allele variances and molecular epidemiology showing the SNP influence on disease risk, which of course is why we're here today, because we have that kind of evidence.

So let's hope I can advance this.

[SLIDE CHANGE]

DR. GINSBERG: Yeah. That time it worked. All right, so just as a broad outline, you know, again to level the playing field, the kinds of pathways we're talking about here from carcinogen exposure into a human subject or it could be a rodent as well in terms of our test systems in toxicology. Phase one, oxidation

pathways. Like the cytochrome P450s I listed, there are two of them, which have been studied a fair bit for SNPs. And I'll leave that to Dr. Guengerich to further discuss the CYPs.

And phase -- but that first phase of oxidation of many of these xenobiotics leads to them being more water soluble, but also perhaps more toxic to especially the local systems, like the liver, where a lot of this oxidation occurs. Phase 2 conjugation pathways, which are often involved in detoxification of those oxidized metabolites. So there, we have the n-acetyltransferases, the GSTs, or the glutathione transferases, and UDPGTs, or are the glucuronyl transferases. And those are just a small subset of phase 2 conjugation, but these have been well studied as well for their variance, and all of these could potentially influence how long a reactive metabolite will have residence time near a target like protein or DNA before they get conjugated and removed from -- towards the kidney and excreted.

I have another arrow leading to detoxification. Some people will still categorize these as phase 2 conjugation reactions, epoxide hydrolase, and NADPH quinone oxidase, one -- subsets 1 for that family and NQO1 and epoxide hydrolase are further metabolizing steps that can help to decrease the risk from epoxides or from

quinones, which can form endogenously as well as through xenobiotic and entrance into the body.

And then finally, over to DNA repair enzymes, which are known to be polymorphic, I'll talk a little bit more XRCC1, but -- which is a scaffolding gene, which helps to organize base excision and nucleotide excision repair pathways. And then the oxyguanosine glycosylase or OGG and, of course, the famous BRCA1 and BRCA2 genes, which are all known to be polymorphic with influential risk factors there. So what we did in cataloging -- you know, I think that's in the next slide hopefully.

[SLIDE CHANGE]

DR. GINSBERG: Oh. No, I went too far again.

All right. So another -- well, let me just say -- what I started saying, what we did with cataloging these various systems according to phase 1, phase 2, and other kinds of detoxification pathways was we basically looked at the various alleles that have been identified and what the functional effect was. So characterize the magnitude of the change in protein function and then looked at the allele frequencies. And based upon combining these two, we developed population distribution of enzyme function that could be used in a risk assessment.

All right. And again, for some more level

setting. Genetic polymorphism by definition is a frequency of at least 1 percent in the population we're talking about. So if it's less than that, it's considered more of a rare genetic change or defect. There can be some examples. Our key deletion polymorphisms. For example Dr. Vasiliou will talk about aldehyde dehydrogenase-2, which is highly influential in terms of some folks not being able to tolerate alcohol at all or if you're heterozygous for the gene that leads to an increased risk of gastrointestinal cancer, if you drink alcohol.

The glutathione transferase M1 -- subfamily M1 and T1 genes, which have actual null polymorphisms, again these are deletion polymorphisms, aldehyde dehydrogenase-2, GSTM1 and T1. These null or deletion polymorphisms essentially ablate the function. It's like a knockout deletion or knockout mutation. So it takes that person, especially if they're homozygous for the allele for the -- for the genotype, no function of that pathway in any of the cells in the body.

NQO1 also has a critical deletion polymorphism we'll talk more about. And then NAT2 is not a deletion polymorphism. It just slows down the function of the protein.

[SLIDE CHANGE

DR. GINSBERG: Those are a couple of examples. You know, I just want to do a little side-step here to an example that we talked about in Science and Decisions, the 2009 update to the red book from the National Academy on EPA risk methods. And in that, we refer to this 2007 paper by Demchuk et al. And I just want to show it as sort of a conceptual piece that talks about 16 different asthma susceptibility genes that have been identified through occupational studies to -- with various -- exposure to various occupational allergens and that each one of these individually has an elevated odds risk for occupational asthma. But if you theoretically combine them all, in other words, if one person had all 16 of these traits, what do you get?

And that's the next slide.

[SLIDE CHANGE]

DR. GINSBERG: And here is -- this slide didn't quite come out right. But anyway, I think you'll get the drift that if you are the wild type for all 16, that you have an odds ratio of close to 1 for occupational asthma where the arrow is at the top left. If you have a fair number of these compile -- compiling variances. So, for example, for an odds ratio of a hundred for occupational asthma, you're at about one in a million likelihood that that individual actually exists.

So, you know, in a large worker cohort, you might have one person that has an odds ratio of 100. According to this theoretical framework for understanding how polymorphisms may multiply their effects or interact their effects within the same host. And we'll talk -- we'll -- that kind of example I'll get to in a minute when it comes to benzene and carcinogenesis in the Chinese population.

[SLIDE CHANGE]

DR. GINSBERG: All right. And here's a bit of our cataloging. This is one paper we published in Journal of Toxicology and Environmental Health. And here's an example of what we were able to summarize for each of the pathways we focused on, which were 11 different pathways and we identified the various alleles that look like they're influential. We talked about -- you know, we kept -- we brought together the evidence on how large a functional effect these -- or inducibility effect these alleles would have on these various enzyme systems.

And then we -- unfortunately, I don't think I know where the pointer is on this, but the third -- one, two, three -- the fourth column over shows the allele frequency in Caucasians, and then in African Americans, and in Asians, so that we have the basic information needed to then do Monte Carlo analysis and come up with a population distribution of protein -- of enzyme function

of each one of these genes.

And so when we did that, here is an example where we looked across these various -- and by the way, we didn't just pick genes to study and proteins -- and enzymes to study, because, you know, for -- just because they're out there in the literature, but these look like they're important pathways in cancer and noncancer toxicology. And when you just look across the variability introduced by polymorphisms, here is a graph showing the percentage of the population, again Caucasian, African American, or Asian, the percentage of the population, which is more than 3.2-fold or half a log different, which is sort of a standard toxicokinetic assumption in risk assessment, half a log for toxicokinetic variability. This is more than that standard assumption because of these polymorphisms.

[SLIDE CHANGE]

DR. GINSBERG: And then the next slide is 10-fold. So now, here's percentages of the population where, for example, with GSTM1, or T1, you can see in the Asian population that 40 to 50 percent of the population would be expected to have at least one allele that could confer a -- a 10-fold difference from the -- it should say median, not mean there -- from the median activity for that enzyme. So again, showing the relatively important

influence of polymorphisms on detoxification in the glutathione transferases or detoxification enzymes, n-acetyltransferases usually thought of as detoxification enzymes.

And I have in this slide different substrates, because it's -- the story can change a little bit depending upon what the researchers used for the substrate to probe the function of the gene, so just trying to be as transparent as possible.

[SLIDE CHANGE]

DR. GINSBERG: And if we look at what we know to be vulnerability or susceptibility polymorphisms in the GSTM1 and GSTT1 -- and again the pathways -- and these pathways are very important to help glutathione detoxify electrophiles, many of which may be mutagenic and carcinogenic, we could see that based upon the frequencies of these polymorphisms that in Caucasians, Mexican Americans, and African Americans, the double null, which is the striped bar, the shortest bar in each set, but the double null runs say less than 10 percent.

So in other -- these can kind of M1 and T1 can fill in for each other. So if you have both knocked out, your risks should theoretically be higher than if only one was knocked out for say electrophiles. But then if you look again at the Asian populations we had data for at

that time, the rates are more like 15 to 30 percent of the double null polymorphism.

[SLIDE CHANGE]

DR. GINSBERG: And now, let's talk a little bit about NADPH quinone reductase -- oxidoreductase, so -- and benzene a little bit. And it's a key defense against benzene hematotoxicity and bone marrow or leukemia -- bone marrow toxicity and leukemia. And so the way the enzyme works is that it helps to reduce quinones. And so when an oxidized version of benzene or phenol becomes oxidized to a double oxidation step, as you could see in that first structure there, phenol, with two double bonded oxygens to it, and if it gets partially reduced, you get into a vicious cycle with it, where it could form oxidative radicals, which can damage proteins. It could go back or it could actually, in the second step, form superoxide, and again induce more bone marrow damage that way and cause hematotoxicity that way or lead to leukemia.

But the action of NQ1 on the bottom part of this slide is to provide a 2 electron reduction of the quinone to form phenols -- a biphenol which is much less toxic and easier to conjugate and eliminate. So NQO1 in bone marrow is an essential defense mechanism against benzene, hematotoxicity, and carcinogenesis. And it has a null polymorphism as well, where some individuals do not have

that enzyme function.

[SLIDE CHANGE]

DR. GINSBERG: And here's the frequency of that across a range of groups. And again, unfortunately, the Asian population tends to be higher with that trait -- allele frequency.

[SLIDE CHANGE]

DR. GINSBERG: And then how do we combine our thinking on these various pathways and how they may interact in a single person? And again, we talked a little bit about the theoretical risk for occupational asthma with -- you know, earlier, but this is now looking at the subject of today's presentation on cancer. And we -- this study from Chen et al. 2007 looked at 100 Chinese benzene workers, 100 with chronic benzene poisoning and 90 that had no evidence of low white blood cell counts or platelet counts, so no evidence of any bone marrow damage. And when they looked at the pharmacogenetics of these 190 workers.

[SLIDE CHANGE]

DR. GINSBERG: And benzene occupation -- just a little bit of background on benzene, roughly 3 -- as of 2008, roughly 3 million U.S. workers in various industries, the human leukemia evidence is strong from rubber-related workers, solvent-related workers. NCI and

the Chinese Academy of Science have done a number of studies on over 30,000 workers in China, an elevated leukemia rate, even below the occupational standard at that time of 10 parts per million. And the risk is highly variable across workers. And so the goal was to try to understand what's the source of that variability for similar exposures.

[SLIDE CHANGE]

DR. GINSBERG: And so this paper again looks at the effect of genetic polymorphisms on the risk of chronic benzene poisoning. And I introduced several of these polymorphisms on previous sides. I'll quickly go through these. And the last column, the adjusted odds ratio for the bone marrow damage, the hematotoxicity is what we're -- the endpoint we're looking at here, not cancer, but something that could be related to cancer, because we're in the bone marrow. We're being toxic to bone marrow cells through a benzene-related pathway. And if you're not killing the cells, you're likely still mutating them and potentially leading to increased leukemia risk.

And so for the NQO1 common variant, which goes -- at nucleotide 609 C to T transition, the TT, the variant, had an odds ratio for increased hematotoxicity of nearly threefold. That's the right most column. The third -- unfortunately my pointer is not working. Let's see. No,

I still can't get the pointer to work, but that 2.94 number is -- I'm going to draw your attention to that last column.

So that's the NQO1 polymorphism by itself. But these researchers have also studied myeloperoxidase, which is primarily a bone marrow oxidative pathway. There's a polymorphism there that did not produce by itself a statistically significant response, CYP2E1, which did not produce a statistically significant change. But the glutathione transferase, which I've already talked a little bit about going from non-null, or the wild type, to the null variant produced almost a doubling in risk by itself for -- in these 190 benzene workers.

So that was a fairly influential gene. The GSTM1 by itself also not quite statistically significant, but in the direction of increased risk in those who are null for that. So let's see what happens when we interact these pathways?

[SLIDE CHANGE]

DR. GINSBERG: So I'll draw your attention not to the top graph, but to the bottom one in the interests of time. So when you interact the NQO1 polymorphism, the GSTT1 polymorphism, and the GSTM1 polymorphism and look at the genetic susceptibility to chronic benzene poisoning in these 190 workers, the -- basically the bottom line is the

top line in Table 5.

And you can see that 20.4 adjusted odds ratio for -- so 20-fold higher risk for this outcome, if you had all three. So the more -- the risk gene or the knockout Gene for NQO1, the knockout gene for GSTT1, and the knockout gene for GSTM1. So 20-fold higher risk.

[SLIDE CHANGE]

DR. GINSBERG: So that just -- let's see if I could back that up one second.

No. I went the wrong way. Sorry, I'm struggling with the mouse. Okay. It's just on a delay. I should -- I need to learn patience.

All right. Well, let's stick with this. So following up on that, they did not study XRCC1, which is a base excision repair polymorphism. And it's known that there's decreased function of that with certain variants. And here's work that I was involved with looking at the percent change in enzyme function for two different genotypes. And the more influential one is the one on the right, the arginine to glycine at nucleotide 399. And you could see that there is epidemiology evidence for increased NNK, or -- that's a tobacco-related carcinogen sister chromatid exchange is in smokers, benzo(a)pyrene, diol epoxide, DNA-related breaks, DNA breaks -- I forget exactly what the endpoint was there.

So those deflections are positive for influence of that gene. The negative deflections are also in the direction of increased risk for a variety of other pathways. So to show that this DNA XRCC1 variant, which is decreased function of that DNA repair gene, leads to increase just all by itself without interacting it with phase 1 or phase 2 pathways, but now we're looking at DNA repair. Polymorphisms can also lead on their own to increased risk for DNA damage.

And waiting for the slides to change.

[SLIDE CHANGE]

DR. GINSBERG: Here's the frequency of the XRCC1 genotypes. And you can see they're fairly common, roughly 10 percent of Caucasians and Asians are about 10 percent where they are homozygous for this low metabolizer phenotype, roughly about up to a maximum of about four fold in the repair proficiency for some of these DNA damaged effects.

So where does this leave us?

[SLIDE CHANGE]

DR. GINSBERG: So Science and Decisions that Dr. Zeise and I worked together on that committee talked about the variability considerations for carcinogens. And there was a case study presented in that by Bois et al. 1995 on 4-aminobiphenyl, where the variability -- this was without

considering polymorphisms, but this was just looking at the population variability and what's known for enzyme function. And the Committee found that 16- to 25-fold difference between the upper bound and the median function of the -- or the likelihood for the production of the n-hydroxyaminobiphenyl pathway. You could find that level of variance in the population.

So the NIS concluded that a factor of 25 would be reasonable default value to assume as the ratio between the median and the upper 95 percentile person's cancer sensitivity for an enzyme -- this is just now one enzyme pathway for bladder cancer for the variability that's inherent in the population.

[SLIDE CHANGE]

DR. GINSBERG: So following up on n-acetyltransferase 2, and another aromatic amine, this is an example from 2023, a paper by Habil et al., and they looked at slow acetylator, rapid acetylator for forming n-hydroxy AA or n-acetyl AA. And, you know, the basic aromatic amine story is that if you first n-hydroxylate and then acetylate, you get more DNA damage and mutations.

If you first acetylate that can then lead to additional conjugation reactions and detoxification. So if you first n-hydroxylate the aromatic amine and then acetylate it, that's the greater risk pathway. And this

research looked at several variants of an N-acetyltransferase 2, subtype 2, and the star 4 pathway, star 5B pathway, or star 7B pathway and the increased risk over on the right graph about a threefold increase in mutations in that genetic subtype of the 7B variant. And the 7B -- the NAT2 7B n-acetyltransferase 7B variant is an intermediate. It's not super slow as -- 5B is the really slow enzyme variant. So it's not quite null knockout, but it's much lower activity. The 7B has intermediate activity and it leads to increased risk as compared to the wild type.

[SLIDE CHANGE]

DR. GINSBERG: And here is a summary of NAT1 and NAT2 polymorphisms that we published in 2009. And for the NAT2 allele, the 7A and the 7B, which is that intermediate function for n-acetylation, o-acetylation is in the middle of all those variances that we looked at.

[SLIDE CHANGE]

DR. GINSBERG: And so again, it's not super slow. It's not as fast as wild type. It's an intermediate risk factor, but -- so you wouldn't necessarily call it out or predict that it would be particularly influential, but it is. And the NAT2 polymorphism overall when you look at the gene frequencies for various functional levels of NAT2 in Caucasians, African Americans, and Chinese, you get

these Monte Carlo based plots of the function of these pathways. This is using caffeine as the indicator.

And again, a lot of the probe substrates are, you know, relatively innocuous that you can give to groups of people and look at their metabolites in urine, and you can see these kinds of -- again, here for NAT2, you get largely a bimodal function of the enzyme.

So the bottom line for this is that, you know, again, if I had the pointer, the caffeine metabolism ratio would be really way out on one tail on the right tail for the -- for -- well, it's -- the 7 -- the highly risk -- the high risk NAT2 polymorphism, 7B and 7A, would not be way out at the right tail. It would be more in that second lump -- hump for slower enzyme function. And yet, it seems to be the most -- the highest risk factor. That's -- it's that intermediate NAT2 function, which does allow n-hydroxylation to occur, but then it will then acetylate the n-hydroxy quite actively.

[SLIDE CHANGE]

DR. GINSBERG: So NAT2 subtype 7 confers 2.5 greater mutagenicity. And it's a homozygote in 1 to 2 percent of the population. So it's not a real large percentage of people walking around with this homozygote, but it does look like a significant risk factor.

[SLIDE CHANGE]

DR. GINSBERG: So to sum up, proposed framework for assessing variability in cancer susceptibility genes due to single nucleotide polymorphisms. Here's a stepwise approach, identify the key enzymes, transporters, binding proteins in a chemical's adverse outcome pathway, evaluate the effect of SNPs at each step, gather in vivo and cell culture evidence for the most influential SNPs, evaluate population distribution of these influential SNPs.

Then you can do Monte Carlo analysis to establish the distribution of risk phenotypes based upon these underlying genotype frequencies, and consider multiplicative risk across multiple SNPs, as for example the benzene NQO1, and glutathione transferase interaction.

Check predictions against molecular epidemiology studies, where they've actually looked at a number of these pathways, and also you can check results against the full PBPK model, because this can all be modelable through physiologically based pharmacokinetics.

[SLIDE CHANGE]

DR. GINSBERG: And then just the final slide. Some thoughts for how to think about this for policy. If the framework leads to the conclusion that SNPs likely increase cancer vulnerability perhaps two approaches, the general population approach consider using the NAS default, which is roughly 25-fold greater risk at the

90th -- 95th percentile upper tail of the pop -- of risk distribution of the population, so meaning about a 6.8-fold increased risk from median.

So in other words, if you have an epidemiology study that does not look at polymorphisms, and you've got a certain risk level, you might think that if you're aware that there are underlying polymorphisms that can push risk in the wrong direction, you may, just as a default, think about an increased risk for carcinogen at an upper tail of about 6.8-fold that you may want to consider in your risk assessment or to be a little bit more analytical about it, you can look at subpopulation specific risk assessment based upon the magnitude of the excess risk and the size of the at-risk population.

And so perhaps this is just a straw man, you might consider doing a separate subpopulation risk assessment when there's at least a twofold excess risk in 5 percent or more of the population, 10-fold excess risk in one percent or more of the population, or a 100-fold excess risk in 0.1 percent or more of the population. So just different ways to think about whether it's worth doing a subpopulation risk assessment. Again for the benzene example, we had a 20-fold excess risk in about 10 percent of the Asian population. So that in this framework would merit doing a separate risk assessment on

that.

So thanks a lot and happy to be part of a panel or answer any questions

CHAIR LOOMIS: Thank you, Dr. Ginsberg.

(Applause).

CHAIR LOOMIS: That's a very interesting presentation.

We have just a few minutes now for clarifying questions. I'll ask the Committee to hold substantive and theoretical questions for later when we have time for that, but are there any clarifying questions right now?

Looks like there is one.

COMMITTEE MEMBER EASTMOND: I have one.

CHAIR LOOMIS: Dr. Eastmond.

COMMITTEE MEMBER EASTMOND: Nice talk. It's my understanding that with the NAT2 variance, there are many different alleles. So you picked up the star 7. There are like 20 or more of these. Is that one particularly of concern or would you do this with each one of those?

DR. GINSBERG: Yeah. So there is enough literature to show that the 7B is influential. The problem with it is is that it's not that frequent. So it's hard to study in epidemiology studies. But when there have been in vitro analyses to show the theoretical increase in DNA damage, the 7B allele turns out to be

highly influential. So some of the others like the 5B, which is much less frequent, is not as influential in terms of outcome of DNA adducts or for the aromatic amines.

So that's -- that one is sifting out right now.

CHAIR LOOMIS: Any other questions of clarification at this point?

Okay. Seeing none, thank you, Dr. Ginsberg.

We'll move on to our next speaker. He's Dr. F. Peter Guengerich. He's Chair and professor of biochemistry at Vanderbilt University. He's an enzymologist with interests in the characterization of cytochrome P450 enzymes, and metabolism, and bioactivation of drugs and toxic chemicals. He's published 768 refereed papers, 324 invited reviews, and 138 published proceedings and is one of the most highly cited authors in the fields of biochemistry and toxicology.

Dr. Guengerich, the floor is yours.

(Thereupon a slide presentation).

DR. GUENGERICH: Oh. Am I on now?

Okay. I think I'm going to stand over there. I sort of like being over -- I don't want to be a lawyer. No offense to your lawyers in the crowd. But at least all -- I can see all of you and we'll proceed with this.

Okay. So anyway, I'm Fred Guengerich. And it's

nice to be here today and I'll try to shed some light or maybe some confusion on where we are today at least bring up some of the caveats about what we have and see how fast this is.

[SLIDE CHANGE]

DR. GUENGERICH: Okay. So the concept that there's a genetic variation related to disease is not really a new one. This goes back to a, you know, famous book about the inherited basis of metabolic disease, going back to at least 1960 with Jim Wyngaarden and Don Fredrickson. So it's not really new.

[SLIDE CHANGE]

DR. GUENGERICH: And I'm not going to dwell on this. This is toxic pathways. It's a slide I've used in some of my course work for teaching toxicology. It looks complicated, but this is actually a gross oversimplification of how complicated life really is in terms of understanding things. One of the things though that is important up there is metabolism at the top leading to differences in reactions with receptors, also covalent binding, mutation, et cetera, and we have a bunch of other things going on. But it's -- I think if there's one thing we've learned during my career in the field it's that there's no single one target to get toxicity. There are a lot of ways to get toxicity and cancer for that ma

matter.

[SLIDE CHANGE]

DR. GUENGERICH: Here are some bioactivation reactions. I won't really dwell on this. Many of you are familiar with these just showing you some of the chemical transformations, primarily using cytochrome P450 enzymes. I think one thing to point out here is that in many cases we have not only -- and this, you know, is something already brought up in the previous talk. You all often have multiple enzymes involved in a pathway. So you -- for instance, with to 2-aminoflourene. You have sulfotransferase there with benzo(a)pyrene. You have epoxide hydrolase, et cetera. So we have a balance of a bunch of enzymes in most of these pathways that actually influence the overall toxicity.

[SLIDE CHANGE]

DR. GUENGERICH: So if we look at bioactivation, this is from a couple reviews that my friend Slobodan Rendic and I have written. And I don't know if you can see these very well, they're different reactions. These are bioactivation reactions. We've made different pies for detoxifications as well, but this is a bioactivation. So there are a bunch of different types of reactions and there are also enzyme families involved in this. The main point I want to make is that if we actually look at the

P450 slice of the pie, it accounts for about 70 percent of the bioactivation, so that's why I'm here. It's probably why you asked me to come and talk about cytochrome P450.

Then if we break down the cytochrome P450 pie in terms of the human enzymes that we know are involved or at least the ones where there are literature reports that we gleaned, you can actually see the breakdown. And about half of these are actually done by Family 1 enzymes, which I'll mention later. This is not -- this is basically a slice at this time in history. And as literature changes over the years, maybe this will change too. But right now, we see the Family 1 involved a lot, but also others.

[SLIDE CHANGE]

DR. GUENGERICH: So what about cytochrome P450 or sometimes called CYP. You know if we actually -- it's a pretty big business. This is something I put together for a P450 meeting. There are close to 80,000 P450 papers in the literature. There about 3,000 a year that are being published. And right now, we're up to 650,000 genes then. And at least 850 of these have some kind of crystal structure then. So basically, we have a lot and it's growing, still continuing to grow in the field. There are only 57 human P450 genes by the way. It turns out that plants and even some lower things like C. elegans actually have many more genes than we do as it turns out then. By

the way, I started in this business in 1973, so it's changed a lot.

[SLIDE CHANGE]

DR. GUENGERICH: I didn't expect to be in this all my life. Anyway, one of the way of taking the 57 human P450s is to -- you know, split them up based on their major substrate class. About a fourth of them are actually pretty essential and are involved in steroid metabolism. The ones that are of most interest in terms of potentially toxic chemicals and carcinogens are in the second row, the one called xenobiotics. This includes drugs as well. I'm not going to say too much about drugs today, but the -- you can see some of the culprits there in the xenobiotics group then. So those are the ones that have been of most interest.

[SLIDE CHANGE]

DR. GUENGERICH: Okay. There are two aspects of safety assessment, hazard identification and risk assessment. Dr. Sun told me not to talk too much about risk assessment, because that wasn't the interest today, but we really can't have one without the other, but I'll focus on -- I'll skip this.

[SLIDE CHANGE]

DR. GUENGERICH: This is risk assessment showing dose response curves and overlap.

[SLIDE CHANGE]

DR. GUENGERICH: And this is BMDL approaches, which I'll skip as well too.

[SLIDE CHANGE]

DR. GUENGERICH: So going back, and I skip over this, this is -- some of these things I think have already been covered by Dr. Ginsberg. One of the things is if you actually look at a distribution of -- frequency distribution of some kind of an effect in a population, if it's unimodal, that tends to argue against genetics, although not necessarily. If you see a bimodal or trimodal distribution, that's a real telltale sign that you actually do actually have some kind of genetic basis for that. There are a number of ways to establish this. People have used family studies, twin studies, things like that.

And it may be complicated. I've got a couple of examples down there. Sometimes you have a mixture of both inducibility of an enzyme as well as in genetic variation, sometimes you can actually have genetic variation in the elements that actually control the induction. So I'll get into this later. This is why it's been tough teasing some of these things out of them.

[SLIDE CHANGE]

DR. GUENGERICH: So another point here. As

already pointed out, strictly speaking, and I'm not a real geneticist, but polymorphism, as I understand now, refers to something at greater than 1 percent incidence in the poly -- population. So that gives rise to SNPs. I've pretty much switched over to using SNVs in -- and that basically include just talking about variation in general, because this actually includes polymorphisms as well. So here are some examples you've already heard about. And Vasilis -- Dr. Vasiliou will talk more about alcohol later. You've already heard about n-acetyltransferase. P450s, there's several classic ones and I'll talk about a couple of those. A lot of this has been worked out with drugs actually.

[SLIDE CHANGE]

DR. GUENGERICH: So how do you get a polyvariation then. I won't go through this. Most of the time, you actually don't see these when there are variations. That is I'm going to try one thing here. No, that didn't -- that's the old slide.

Let me go back.

Okay. Whoops. I'll go back.

I'm trying to go back.

DR. GINSBERG: Yeah, I had the same problem.

DR. GUENGERICH: You did. Okay.

Well, what are we going to do about it?

DR. GINSBERG: The left arrow.

DR. GUENGERICH: The left arrow.

Okay. So I think we are here. A number of basic things can hear -- you can actually have a base pair substitution giving rise to a change in an amino acid. You can also have base insertions or deletions. And these usually -- you know, things cause proteins to stop prematurely. You can insert codon and you can have other issues too, including RNA maturation issues, which has actually turned out to be pretty common then in some of the P450 issues then.

[SLIDE CHANGE]

DR. GUENGERICH: Okay. So back in the 1990s, the National Institute of Environmental Health Sciences got all -- or NIEHS, got all excited and started something called the Environmental Genome Project. This was when Ken Olden was still the Director. And this was about the time that it was -- they were about to finish the human sequence.

And so they got the bright idea, well, this is great. This will actually -- we can actually use this information to explain variations in disease, environmentally induced disease. So they started the Environmental Genome Project. And this is blurb I wrote for that. It was published in Environmental Health

Perspectives in 1998. And this all sounded pretty good. So mind you, this was about 26 years ago.

[SLIDE CHANGE]

DR. GUENGERICH: And so we thought -- well, okay, so what's some of the basis here. Well, you've already heard a little bit about DNA repair. And it's very clear that there are some big time variants there that actually make a big difference. And these are very serious diseases then associated with these. I won't go through all of these. They're fortunately not too common. Some of them are probably even embryonic lethal. But basically, there's some really bad stuff happening here. And, of course, up at the top, you actually have environmental exposures. So some of these people who are afflicted with these, for instance, are very, very sensitive to sunlight, things like that. So the -- so that's, you know, a part of the basis for going on with this.

[SLIDE CHANGE]

DR. GUENGERICH: But -- and here's another case. This is in cytochrome P450. It actually deals with genetic issues in cyanide sensitivity. So as many as some people know -- many of you know, some people are extremely sensitive to cigarette smoke. And, you know, they can't even go in a -- well, I guess it's changed now. It used

to be you couldn't go into a bar because they have, you know, really bad visual problems. I guess now you can't -- people can't smoke in bars, definitely in California.

Anyway, this -- a lot of this deals with an enzyme called Rhodanese. And don't go -- that's not a typo. It's not a "ase" it's actually an "ese". I won't go into why, but there are a number of things -- deficiencies known with that, which make people sensitive to cyanide.

[SLIDE CHANGE]

DR. GUENGERICH: This is a -- the abstract from an article -- well, it's an article -- from an article I wrote for Cancer Research in 1988. And most of this was based on animal studies, which we were in full swing by that time. So it turns out that there was a lot of evidence in differences in cytochrome P450 composition, could influence susceptibility to a cancer then. Some of this was genetic. Some of it was inducibility stuff by Jim and Betty Miller at the University of Wisconsin.

And I thought -- you know, I think everybody thought that this -- we were just sort of right around the corner from really applying all of this to humans. So I wrote this review then and asked a number of questions, like down at the bottom, which enzyme reactions are most

relevant to chemical carcinogenesis?

We have -- I think we've actually solved that pretty well, but we still have a lot of problems, and I'll tell you why in a minute.

Also, one of the questions is how adequate our animal models were for prediction of cancer in humans and the influence of genetics.

[SLIDE CHANGE]

DR. GUENGERICH: So this is some work -- an old slide from Dan Nebert, who I'll mention later. He was actually Vasiliou's mentor -- when a -- Vasiliou's mentor -- Vasilis's mentors and he may talk about him too. But there -- it was clearly known you could actually find different genetic strains of mice that were more or less prone to getting cancer. And you could actually induce -- relate that to the inducibility of cytochrome P450 1A1 or actually probably 1B1 as we know now. So this was quite clear. And the question was would this happen with people?

[SLIDE CHANGE]

DR. GUENGERICH: Well, here we are today and the question is are there any real links to cancer yet? This is an old slide, but I'm not sure it's really changed all that much. There's some that people keep kicking around. It's been hard to pin down most of these. There are a few

isolated incidence, but it's been difficult and I'll tell you why. There's still some prospects out there. I'll talk a little bit about 1A2. I'm not going to talk about 2A6. 1B1 is sort of still on the dock.

[SLIDE CHANGE]

DR. GUENGERICH: This goes back. I apologize for the small print. This is a paper from 1973. It's a Shaw and Kellerman study. And this is 1973. Shaw and Kellerman, then at the University of Wisconsin, found that they could actually take lymphocytes from people, from smokers, and basically they were able to relate the inducibility of what's called the AHH response, which is basically cytochrome P450 1 enzymes to whether these people were more likely to get cancer, lung cancer. And they got really sort of a trimodal distribution.

[SLIDE CHANGE]

DR. GUENGERICH: Others went on. This is another people -- paper from Dan Nebert. It turned out that this was technically very messy. People started doing more and it turned out it depended on what time of the year you actually harvest the lymphocytes from people and things like that, and it got to be very messy, and people were kind of wondering about it.

[SLIDE CHANGE]

DR. GUENGERICH: So going on back now into the

1990s. This is work from Tetsuya Kamataki's work in Japan in Hokkaido, and he showed that P450 1B1, not 1A1 is the major AHH enzyme in human leukocytes. And this showed this sort of modality too.

So the truth is at the end of the day, even after 51 years, we're not really sure about this and we've been sort of stuck with this thing. And I don't think it's really got there.

[SLIDE CHANGE]

DR. GUENGERICH: Here's another slide and I don't know the answer here. Maybe Vasilis will talk -- well, I don't know if he's going to talk about it. But there's a relationship between P450 1B1 and glaucoma. And he and Frank Gonzalez have done a little bit on this. You can reproduce the defect in mice. But still to this day, and maybe Vasilis can tell me the answer, we don't really know the reaction that's involved here in terms of any of the known substrates.

[SLIDE CHANGE]

DR. GUENGERICH: So drugs. So why do we consider drug toxicology in this course. This is my toxicology course. The -- well, as I tell them, some of these people may be getting jobs in this area, but also -- yeah, there are a lot of advantage of studying the toxicology of drugs, because you actually know what people are exposed

to. You can control that. Whereas, with the things like environmental carcinogens, we really have a hard time even knowing what the carcinogens are in many cases, let alone the dose.

[SLIDE CHANGE]

DR. GUENGERICH: So going into drugs, this is Bob Smith. And this is a story of P450 2D6. And this goes back to, I think, 1977. And basically, he's the one on the right. I'm on the left there.

[SLIDE CHANGE]

DR. GUENGERICH: The two papers, one by Bob Smith's group and the other by Geoff Tucker, and basically they found a polymorphic variation in the ability to metabolize debrisoquine. So the people on the right hand of that graph are slower metabolizers. This is the ratio -- the urinary ratio of the metab -- of the drug to the metabolite. So the bigger the number, the slower the metabolism. And at first they found that they were the two groups, the extensive metabolizers and the poor metabolizers.

Later on, it turned out there are ultrarapid metabolizers, and I'll say more about those -- that in a minute. And so this might look like pretty good then. This was pretty real. And Bob Smith was actually one of the people participating in this trial with the drug.

It's an antihypertensive. And he passed out in the test. The -- they did it again and he passed out again. So he thought that this was kind of real.

So why is this an issue? Well, with drugs, basically, if you're a poor metabolizer, you're not going to metabolize the drug away and the pharmacist or physician will probably prescribe the same dose of drugs, so you won't be clearing it out as fast. And the same thing goes for any other chemical then.

So where are we today?

[SLIDE CHANGE]

DR. GUENGERICH: Well, the -- this is not the latest run down, but it's one in terms of the list of all the 2D6 alleles. Last time I tried to count, there weren't just three groups. There were 160. Okay. And there are probably a whole lot more out there.

Now, the other problem is this, and I'll see if -- okay, yeah, you can see that loop that just came up. It turns out that we actually don't know the effect of most of these, in fact, probably fewer than 10 percent. So you can actually do DNA sequencing a whole lot faster than you can do serious biochemistry and try to find what's really going on, so that's one of the problems.

[SLIDE CHANGE]

DR. GUENGERICH: So here's something that came up

fairly early in the business too. And this is also -- this is from Jeff Idle who is in Bob Smith's group and in 1983 published a Nature paper, so it must be important, of the -- and basically, he -- they were looking at lung cancer. And if you look at the two graphs, the frequency plots, on the left-hand side, the slow metabolizers or poor metabolizers are less likely to get lung cancer than the normal extensive metabolizer. So this looked pretty good and people got all excited and people started trying to repeat it.

[SLIDE CHANGE]

DR. GUENGERICH: And we got interested in this, because one of the obvious explanations would be that there's something in tobacco smoke that is being metabolized by P450 2D6 to an active carcinogen. Well, we started looking for this. And, you know, it's kind of a mess. We got some cigarette smoke condensate and it was really hard to work with, because it kept killing all the bacteria, and -- in the assays, and then eventually, we found something from an extract of that that was being metabolized by 1A2. Everything with 2D6 came out negative. We could not find anything or any difference in our studies.

[SLIDE CHANGE]

DR. GUENGERICH: Well, people went on and this is

about 1998 from Jeff Tucker and others, basically epidemiologists couldn't repeat this or they did and others couldn't. And basically they said we -- people should just give up and basically people did. So there was nothing to this after all those years and all that money spent.

[SLIDE CHANGE]

DR. GUENGERICH: Here's something with a drug, where a polymorphism actually -- or a variation -- well, I guess it was a polymorphism. This is morphine metabolism. And so basically, you know, one thing P450 2D6 does is convert codeine to morphine. So if you're taking codeine for some reason, it's converted to morphine and then morphine, and I think one of its glucuronides or the active principles.

[SLIDE CHANGE]

DR. GUENGERICH: So this is just the same thing too. This has happened here. And so the problem is if you're one of these ultrarapid metabolisms I alluded to a few minutes ago. Basically, these people have I think up to about 13 copies of the gene. They act -- it's something called gene duplication, which is kind of weird. So they have 13 times more enzyme than most of the other people.

[SLIDE CHANGE]

DR. GUENGERICH: So the problem was, it's kind of a sad case here, this is a woman, a mother -- young mother who -- in Canada who was actually taking codeine. She was breastfeeding her child. And basically, she was converting the codeine to morphine too fast, and actually she was okay, but the child died. So it's kind of a sad case. But this I think was pretty clear as to what was going on.

[SLIDE CHANGE]

DR. GUENGERICH: This is aflatoxin. I won't say too much about aflatoxin. This is a summary of some of the stuff we've done in my lab over the years. But basically, one of the end -- one of the reactions here, Cytochrome P450 we'll convert that to an epoxide or actually two stereoisomers. And then that can be hydrolyzed non-enzymatically or a little bit by epoxide hydrolase.

[SLIDE CHANGE]

DR. GUENGERICH: So along comes a paper and this is in PNAS, so it must be important, an epidemiology paper. And they claimed that polymorphism in epoxide hydrolase is affecting cancer then in China. It turns out though that when you actually do the biochemical studies, it turns out we'd known for a long time that the half-life of the epoxide in water at neutral pH is about 1 second.

You can add epoxide hydrolase and you really don't speed that up. So there's really no biochemical basis for that.

[SLIDE CHANGE]

DR. GUENGERICH: Let's talk about heterocyclic amines. And these are the things, if you didn't know that, they're formed in burned foods as a result of pyrolysis. So if you're grilling, all that black crud is full of heterocyclic amines. And these are actually very potent bacterial mutagens and they're also potential human carcinogens. I think they make a couple of the lists for IARC or NTP. And they actually do cause cancer in rodents for sure.

You have bioactivation through two enzymatic steps. There's n-hydroxylation and then you can have o-acetylation or perhaps sulfation and you get covalent binding to cellular DNA. So anyway, it turns out -- let's go back and talk about P450 1A1 and 1A2.

[SLIDE CHANGE]

DR. GUENGERICH: And Fred Kadlubar and I had shown, I guess about 30 years ago, that caffeine is a good marker for this, because P450 1A2 is metabolizing caffeine. You can actually do a urinary test and people will vary about 40-fold.

It turns out that subsequently with some of these Manhattan plots and things like this in genetics, it turns

out that this locus here, which is for the inducibility of 1A1 and 1A2 or the AH receptor basically determines how much coffee you can drink, okay? So that's basically a way of looking at how much P450 1A2 you have.

[SLIDE CHANGE]

DR. GUENGERICH: So it's kind of a mess -- a tricky situation, because we know that P450 1A2 can activate a lot of these chemicals like the heterocyclic amines. This is some -- from some work Rob Turesky and I did. But basically, the human enzyme is an order of magnitude more active than the rat enzyme. So that shows why you sometimes animal studies aren't that great.

[SLIDE CHANGE]

DR. GUENGERICH: Here's some more work from there with one of these called methyl IQx. And you can see that humans vary. Some are a whole lot more active than the rats, even more so than the inducible -- induced rats in this particular study.

[SLIDE CHANGE]

DR. GUENGERICH: Well, Fred Kadlubar and Nick Lang tried to do a bunch of epidemiology and they actually looked at NAT. They also looked at P450 1A2 by phenotyping. And they also looked at how much charbroiled meat people said they consumed. It turned out you kind of have to put all three of these things together to get any

kind of response. So that was only marginally different. So it hasn't been great in terms of answer for what's really important. I hit that.

[SLIDE CHANGE]

DR. GUENGERICH: Are we stuck here?

Okay. So -- okay, so what are the most likely P450 prospects for genetic variation linkage with environmental diseases?

1B1, yeah, there's something with glaucoma, but we don't know if there's any environmental link or not. 1A1 I said a lot already about that. And even after 50 years, that's not really clear. 1A2, the strongest possibility was probably -- of an association was probably with the heterocyclic amines and the burned food. And that's not really holding up.

2A6 I think has some potential. I haven't really talked about that. This is connected with nicotine metabolism. It's -- it may be due to an adversion to smoking due to the handling of nicotine. I'm not sure about that.

2E1 possibly with some of the small industrial compounds and solvents and maybe benzene. Although, there's limited evidence.

3A4 is more of an issue in drug-drug interactions then.

And 3A5 is a cousin of 3A4 obviously. And this is polymorphic. There's a ratio linkage. You have the same issues as with P450 3A4. I don't think there's any strong evidence that the variations in 3A4 or 5 are really linked to any environmental chemicals, but they are for drugs. Over half -- about half the drugs on the market are metabolized by P450 3A4 and 3A5.

[SLIDE CHANGE]

DR. GUENGERICH: And very quickly, I won't talk about alcohol dehydrogenase and aldehyde dehydrogenase. The next talk will. GSTs you've already heard about. About half the people are missing M1, half -- or about a third are missing T1 or at least in Caucasians. There are also possibilities with the UGTs sulfotransferases, sulfotransferases are involved both in bioactivation, and detoxification, and n-acetyltransferase.

[SLIDE CHANGE]

DR. GUENGERICH: Okay. What about non-genetic variations in human xenobiotic metabolizing enzymes?

Well, this is another issue too. And this is why it gets complicated. P450 2E1, you have not only the polymorphisms, but you have induction by ethanol and there's good evidence for a role here in the toxicity of acetaminophen. I don't know if there's good genetic evidence, but certainly with the inducibility. Alcoholics

are more likely to have problems with acetaminophen toxicity.

1A1, 1A2, and 1B1 you had -- definitely have induction by polycyclic hydrocarbons too. So you have to consider any variance in the induction machinery. And 3A4 and 3A5 you have induction by many drugs.

[SLIDE CHANGE]

DR. GUENGERICH: Also, something has been mentioned, transporters. This has all blown up, you know, in the last 30 years or so. There are a lot of defects in transporters. And unlike the P450s, there aren't just 57, there about 500 different transporters in humans. And these definitely make some differences in drug metabolism. And they probably also do with environmental chemicals.

[SLIDE CHANGE]

DR. GUENGERICH: So back to the Environmental Genome Project, which I mentioned before. As I said, NIEHS got all excited about this and had there -- this in their strategic plan. I don't think they're really doing much with this. As far as I understand today, they seem to be all in on the exposome as opposed to environmental genome interactions then. So that's kind of the flavor.

[SLIDE CHANGE]

DR. GUENGERICH: So in the future, here are some more of the problems we have. We have, you know, in vitro

assays. They may or may not be predictive of what happens in people. And we also have the problem of relating animals to humans as well, so it's -- toxicology is hard.

[SLIDE CHANGE]

DR. GUENGERICH: Really. And finally, this is about the end. Going back to Dan Nebert. Dan wrote -- well, it's kind of an autobiographical review and annual reviews in Pharmacology and Toxicology that's actually quite good. And he covers a lot of stuff he's learned and he's older than I am. So each -- here, this is very important. Each patient's response to a drug or environmental toxicant is now considered to reflect the combination of genetics, epigenetic effects, which I haven't even talked about, endogenous influences, environmental exposure to other things and each individual's microbiome, which I haven't talked about either.

So all but the genetics are continually changing. And so this is why it's tough to really tease things out. There are certain -- I'm sure there are genetic differences in people that relate to susceptibility, but we're trying to look at these in terms of these other background and then also remembering paracelsus. It's the dose that's really important.

[SLIDE CHANGE]

DR. GUENGERICH: And I won't go into this. This is just my lab.

Thank you very much.

(Applause).

CHAIR LOOMIS: Thank you very much, Dr. Guengerich. We do have five minutes or so for questions of clarification, if there are any?

One.

COMMITTEE MEMBER BUSH: Just a clerical question. Is Dr. Guengerich's material available in the materials that we were -- had access to? Will the presentation be there?

COMMITTEE MEMBER EASTMOND: It is.

COMMITTEE MEMBER BUSH: It is there.

DR. GUENGERICH: Yeah, I think so. You're welcome to it.

COMMITTEE MEMBER BUSH: Thank you.

CHAIR LOOMIS: Any other questions?

COMMITTEE MEMBER EASTMOND: I have a question.

CHAIR LOOMIS: Yes.

COMMITTEE MEMBER EASTMOND: You mentioned there were hundreds of thousands of CYP genes. That -- am I correct that is there 57 in humans, but all told there were like 600,000.

DR. GUENGERICH: Yeah, right.

COMMITTEE MEMBER EASTMOND: That seems like an amazing number. How is that ever -- how is this compiled?

DR. GUENGERICH: Well -- oh, yeah, well very simply. Well not simply. It took a lot of work. But basically this includes all the species that have been examined. And, you know, we've got -- they're in bacteria. They're in plants. They're in other microorganisms. So, for instance, when you get into plants, all plants have hundreds. I think wheat has something like 1,200. So basically, humans and mammals, I should say, we're kind of consumers. So we have a handful of these to sort of eat up everything we eat. But in plants, you may have a pathway that just makes one color of the flowers that needs a bunch of P450.

So it's all of them. And so basically how do you -- how do you actually know these are P450? There's a signature sequence about -- around these cysteines that binds the heme. So if you see that, boom, it's a P450. These have not all been characterized. I don't know they'll every -- they ever all will be. Yeah, good question.

CHAIR LOOMIS: Thanks.

Are there any other questions at this time?

All right. Seeing none, I think we should make a decision about when to take lunch.

It's 11:30. I don't know if doing it now is an option. But let me confer with Dr. Zeise and the Committee about whether we take lunch now or proceed with the next speaker and then break and come back for discussion.

Thoughts?

CHAIR LOOMIS: Okay. Well, there's the answer. Let's proceed then.

Our next speaker, Dr. Vasilis Vasiliou is professor of Epidemiology and Chair of the Department of Environmental Health Sciences at the Yale School of Public Health, also with appointments at the Yale School of the Environment and School of Medicine. In his laboratory, they utilize state-of-the-art integrated system approaches that includes metabolomics, lipidomics, exposomics, tissue imaging, mass spectrometry, deep learning, and human cohorts and genetically engineered mouse models to induce -- elucidate mechanisms and discover biomarkers and novel interventions for human disease.

Dr. Vasiliou, the floor is yours.

(Thereupon a slide presentation).

DR. VASILIOU: Thank you. Now, you can.

Well, thank you very much. Thanks for the invitation to be here. And I can tell you how stimulating it is to follow Fred Guengerich one of the -- maybe the

top guru on P450s metabolism.

Anyway, I was lucky during my career, I will show you a few slides that I had worked with all of these -- even Bob Smith I still remember when he visited University of Cincinnati and he was telling us the story, about 2D6, but I will go ahead and -- Oops. Can you move the slides?

[SLIDE CHANGE]

DR. VASILIOU: Okay. So here, I'm going to briefly tell you about my academic history, which has to do with environmental exposures and the reason that you invited me here on aldehyde dehydrogenases.

So I started my PhD in University of Ioannina in Greece. And then I followed up with a post-doctoral and Fogarty Fellowship with Dan Nebert then at University of Cincinnati, where we studied the gene-environment interactions, what Fred was saying, on -- but mostly going on aldehyde dehydrogenase P450s. And then towards the end, we converted those to antioxidant systems including glutathione.

After that, I went to University of Colorado, where I became the Director of Environmental Health Sciences and Toxicology Program. And I continue my work on the gene-environment interactions. And I also give more emphasis to alcohol-induced tissue damage.

In 2014, I moved to Yale and I'm the Chair of

Environmental Health Sciences. I still do genome-exposome interactions in obesity, diabetes, cancer, and neurodegenerative disease. And I really liked Fred's comment about the NIEHS reaching from genetic susceptibility to exposome, which is actually I think we should be somewhere in the middle, because we deal -- we still need to identify susceptible individuals or even resistant individuals to the exposome.

That still is my time.

(Laughter).

[SLIDE CHANGE]

DR. VASILIOU: Okay. First paper for -- if -- it was 1948 by Efraim Racker on the aldehyde dehydrogenase. That was the definition.

[SLIDE CHANGE]

DR. VASILIOU: This was the discovery of aldehyde dehydrogenase, which was followed up with -- followed up with another paper, "Essential Role of Thiol Groups on Aldehyde Dehydrogenases." So these were the two major papers that they essentially brought ALDHs into the field followed up with --

[SLIDE CHANGE]

DR. VASILIOU: -- Oh, with Richard Deitrich's work from University of Colorado who passed away in 2018. So this -- there were two papers by him in JBC on the alde

-- describing the aldehyde dehydrogenases. And then I was lucky enough and we published this huge review on ALDH inhibitors, which we just got -- they got so many citations, we just got invited to give an update on this recently. And it's ready to go after I complete the toxicology chapter for Dr. Guengerich toxicology.

(Laughter).

[SLIDE CHANGE]

DR. VASILIOU: So this is the picture with Richard Dietrich. As I told you, I've been really lucky enough in my career to meet with all these people. This is the legend of aldehyde dehydrogenases. And believe it or not, he was really in his late 80s and he was still in the lab working with me. Over there we're doing catalase experiments in aldehyde dehydrogenase.

[SLIDE CHANGE]

DR. VASILIOU: So non-P450 metabolism of aldehydes. So you can see it's missing the P450s in here. So I bring -- this is -- this is a slide actually which we had generated really early with one of my post-docs. And if you look at any of the alcohol metabolism, now they're using the same pattern. This is alcohol converted to aldehyde by aldehyde -- alcohol dehydrogenases. Unfortunately, I do not have the time to go all over. A lot of genetic polymorphisms from this family.

But also another important thing, aldehyde can come back to the alcohol by ADHs, aldose reductases, and short-chain reductases. On the other hand, alcohol can be converted to acetaldehyde with catalase in peroxisomes in the expense of hydrogen peroxide.

So you have the formation of aldehyde. This is the general scheme. So aldehyde in general can be converted to innocuous carboxylic acid by aldehyde dehydrogenases. But also there is a xanthine oxidase and aldehyde oxidase system which can take that to this pathway. And again, today, we'll focus on the aldehyde dehydrogenases.

[SLIDE CHANGE]

DR. VASILIOU: So this is another scheme that I really like it. And one of the things that I was really fascinated with aldehyde dehydrogenases is their function as an enzyme. So the reaction is very simple. They take a lot of aldehydes which are present in environmental -- in the environment, as you know, biotic metabolism, lipid peroxidation. And they can convert, as I said, to carboxylic acid.

Now, some of those carboxylic acids are very essential, such as retinoic acid. It is a very big factor in murine Development or betaine, which is involved in osmoregulation. So what happened if aldehyde dehy -- if

aldehyde does not get metabolized. Well, complications here are it's capable of covalent binding, inducing further lipid peroxidation and causing your major antioxidant glutathione depletion in there.

And as an effect, you have protein DNA adducts. You have membrane distractions or the lipid peroxidation decides the membranes. You have oxidative stress, and then you have toxicity, and you have disease.

I apologize. It's --

[SLIDE CHANGE]

DR. VASILIOU: anyway. I -- so another thing that I was fascinated with this -- with this gene family or super family is that we were the first to show that some of those aldehydes they also have non-catalytic activities. In for the catalytic activities, we describe what it is. But for the non-catalytics is they can direct -- the can act directly as direct antioxidants, they combine to react to oxygen species, and it's just like glutathione.

They can also absorb UV radiation. And we have shown that with mouse models in the eye where they protect the entire eye structure. And they are also binding to endogenous molecules in there. So multiple functions of the ALDH.

But one of them --

[SLIDE CHANGE]

DR. VASILIOU: So what triggered me to go into that was a gene that I actually -- believe it or not, I discovered this gene before the Genome Project through the -- if you remember the express sequence tags, the ESTs. So we figure out there was this aldehyde dehydrogenase. We called ALDH16A1, which was lacking the catalytic cysteine 302 in humans and, you know, in animal species. And what triggered me on that was, at that time, there was a big review about the dead enzymes. And what dead enzymes was the kinases. As you can see from the nice scheme over there, there were -- you know, they're losing the catalytic activity and they perform as something else.

So on the right-hand side, I don't know if I can point. I don't think the pointer is working. On the right-hand side, you can see how the clusters -- this is evolutionary. Divergence of the genes you can see in the, what I call, higher animals, all the ALDH16A1 they have lost the catalytic activity.

On the other lower animals and in bacteria, they do have catalytic activity. The only exception was the frog. So what happened during the evolution, this ALDH lost the catalytic active site and they perform a particular form -- function, which is independent of the

function.

I put my computer here.

[SLIDE CHANGE]

DR. VASILIOU: Anyway, so this is the Super family. This is aldehyde dehydrogenase, what I call super family, 19 human genes. And this is how they classed it based on the amino acid similarity. And this is -- you can see there are distinct chromosomal locations. However, they are supposed to be some gene duplications like the 3A1 and 3A2. 3B1, 3B2. They're adjacent to chromosome 11 -- 17 and 11. But all the other ones have distinct phenotypes.

Why they're important and how you can say that these enzymes are important? Look at your right-hand side, you can see what I call the mutational phenotypes. And there are a lot of mutations which are associated with -- there a lot of mutational phenotypes -- there are a lot of diseases which are associated with mutations on these genes. And this is very distinct.

So what I have decided, I'll show you one or two of them, but then we will focus on aldehyde dehydrogenase 2 and in response to carcinogens and carcinogenesis.

[SLIDE CHANGE]

DR. VASILIOU: Where is the computer for that? Is it -- maybe -- all right. I'm restricted.

So type II Hyperprolinemia, it's an autosomal recessive disorder associated with seizures and mental retardation. I actually have to tell you most of these ALDH mutations are associated with CNS dysfunction.

In this patient, we have 10 to 15 times higher proline plasma levels, 10 to 40 times higher pyrroline 5-carboxylate level -- plasma levels. And the mutations have been associated on 4A1. You can see, this is the proline arginine metabolism, and this is where the enzyme is. So if the enzyme is not there, you have the higher levels and they can cause all this -- all these issues.

How did you guys manage to change your slides?

[SLIDE CHANGE]

DR. VASILIOU: Okay. Oops, I'm sorry. Oh, boy.

Hydroxybutyric aciduria, again another autosomal recessive trait discovered in 1981. This is on the -- characterized by again retardation in psychomotor and language development, hypotonia, and ataxia. This is accumulation of 4-hydroxybutyric acid and GABA. You can see the metabolism of GABA in there. You can see the metabolism of ALDH5A1.

For both of the small dose, we have knockout models that, you know, this mechanism has been identified in great details.

[SLIDE CHANGE]

DR. VASILIOU: Sjögren-Larsson Syndrome, another very important syndrome, which is due to the microsomal mutations in gene, including the microsomal ALDH3A2, which is involved in the fatty aldehydes -- fatty -- that are coming from fatty alcohol. And this again coming to leukotriene metabolism. And this is very important. We have -- really, people have identified and we have done also find the problem.

So the mutation associated again mental retardation, spastic di- and tetraplegia, chronic ichthyosis, so it's like you have the fish scaling in your skin and also you have macular dystrophy.

So this is the three that I have chosen to show you regarding the changes into the endogenous pathways that Fred was talking about. It's not only the environmental, we have also endogenous. If I can be able to change the slide.

Oh, can you bring me the computer? Oh, that would be perfect. Okay. Perfect. Thank you. Perfect.

[SLIDE CHANGE]

DR. VASILIOU: All right, so aldehydes. Aldehydes potent electrophiles. Again, our toxicology classes, aldehydes aremolecules with really high electrophile potency. And this is just from a recent review that I found in Chemical Research in Toxicology,

which it was more, you know, easy to show you. So on the left, you can see the different aldehydes. On the right-hand side, you can see all the sources that those aldehydes can be generated. And they can be generated from plenty of sources including drinking, smoking, fumes, food sources, industrial, cosmetics, and, of course, don't forget the endogenous. And of course, you do have the direct metabolism as well.

[SLIDE CHANGE]

DR. VASILIOU: So here is -- we have a nice review. And I provided this review for your -- also is the non-P450 aldehyde dehydrogenase enzyme. I heard from people that they really like that, because we describe all the -- all the NA -- the reactions catalyzed by those aldehyde dehydrogenase, but they have taken one to show you, which indicates that many aldehyde dehydrogenases, they can work to metabolize to get rid of one of the aldehydes. This is malondialdehyde on the left, as you can see, ALDH1A1 and 2. And you go to malonic semialdehyde. And then you have also the formation of the acetaldehydes going acetate. And you have a bunch of aldehydes involved including the 16A1 in there. So you can see a bunch of aldehyde dehydrogenases can be involved in the metabolism of a particular molecule. Now, malondialdehyde is also formed during lipid peroxidation

and this is rather important.

[SLIDE CHANGE]

DR. VASILIOU: So this is a scheme that I was telling you. This is what I have spent my life on that in terms of alcohol metabolism. On the right-hand side, you can see my first paper as a graduate student, "The Mechanism of Alcohol Intolerance Produced by Therapeutic Agents." This is what we call the disulfiram reaction. And disulfiram is a drug that you can take and inhibits ALDH2. It has been used to prevent the alcoholism because it makes you feel really bad. However, I can assure you disulfiram is a very nasty drug causing a lot of changes also in Cytochrome P450s and also Phase II enzymes.

Anyway, again, the scheme, I'm not going to go into there. What I want to draw your attention is that the acetaldehyde -- the one I introduced, acetaldehyde can cause DNA and protein adducts. And also, you have during the metabolism of P450s, which Fred was talking about, you have the generation of reactive oxygen species, glutathione depletion, and oxidative stress.

So again, if you have this ALDH, the ALDHs can really help in blocking all these effects from one standpoint. And the second ALDHs can metabolize acetaldehyde to acetate, which then it's converted to acetyl coenzyme A, which then is taken by the Krebs cycle

and can go further down.

Now, acetaldehyde -- acetyl coenzyme A, of course, it can cause epigenetic changes, which may have an effect into the cancer incidence. But again, we want to focus on these changes.

[SLIDE CHANGE]

DR. VASILIOU: So everybody knew that ALDH2 is the major enzyme on metabolizing acetaldehyde. And this has a very low Km, less than 5 micromolar. And by the way, the polymorphism on ALDH2 is the most well studied and the best known polymorphism that we know so far. And we know based on epidemiology and everything.

So when everything was started, I knew from the literature that, of course, we had ALDH1A1, which has a what we say a higher affinity, like a 50 to 100 micromolars for acetaldehyde, but then it was described this enzyme A actually back in the old days, it was called ALDH5 or ALDHX. So we've got the cDNA from the ESTs actually and then we cloned the gene. We expressed the gene and we found, yes, that the ALDH1B1 is metabolizing acetaldehyde and nebulize also the other aldehydes.

And I'm really proud to tell you because this gene -- and unfortunately, we don't have to tell -- time to tell you all this. This gene we found that this is a biomarker for colon cancer. And indeed, our studies were

followed with genetic experiments that showed that this was actually the case. So ALDH1B1 is a determinant for colon cancer. And actually, there is a new paper just came out that ALDH1B1 can bind to a virus as well. This is what I was telling you before.

[SLIDE CHANGE]

DR. VASILIOU: So this is very important.

So let's go to the human ALDH2 alleles. We're talking about SNVs or SNPs before. And I just want to show you how complex is our DNA. I was just playing again with the databases on the 25th. There are 18,788 single nucleotide polymorphisms in the database for ALDH2.

Do you know which one has the sig -- only clinical significance? Only one, ALDH2*2. And the ALDH2*2 is just an amino acid change that is associate -- essentially cause the lack of activity. And these are responsible of what we call, "the flushing syndrome."

What you can see on the right-hand side is a colleague when I was a post-doc in Dan Nebert's lab. On Friday afternoon, we had happy hour. And back in the old days, we did not have cell phones. So I had to take my car, go home, take the camera and come back.

So this is a picture before and after just a little -- a little thing of beer. So these individuals with this mutation they -- the face becomes really red.

And if I can give you another lecture. If you try to make the combinations between AD -- alcohol dehydrogenase polymorphism, which lead to a faster formation of acetaldehyde, and then also the ALDH blockage that you get this fully flushing syndrome.

The problem with that is, and you have to be careful, if you force yourself to drink more, you can die from acetaldehyde toxicity. Die. I mean, coma. You go to coma and you die.

Now, the problem with that, and as we'll discuss is, that if people -- they have one of these alleles they metabolize less. And if they're forced to drink or if they drink, they have higher risk, not only for GI, but for upper digestive cancers. And I show you -- I present you some of the evidence.

[SLIDE CHANGE]

DR. VASILIOU: So a little bit more on this to show you why this -- and this is, as I said, the most well studied polymorphism in terms of the protein. So Henry Weiner in Purdue had done a lot of work on that. The enzyme is a tetramer. As I told you it's one amino acid change. The ALDH2*2 allele is dominant, which means even if you have one copy, all your enzyme is completely inactive. And this is because it changed the conformational, the site where the NAD binds and

essentially makes this catalytically inactive.

Even heterozygotes, they have 6 percent of the wild-type activity. So, you can see the allele frequency in eastern -- or what we call eastern Chinese and Japanese. The Japanese actually have the higher frequency, Chinese, Koreans, Taiwanese. Africans, 0; Caucasian, 0; Native American, 0; worldwide is 7 percent.

However, these numbers will start changing. More people from Asians start getting married with Caucasians. So then we're going to have a penetrance of this allele we're going through and it's going to happen and it does happen.

However, what I want to draw your attention to is very low incidence of ALDH2* allele in alcoholics. However -- in alcoholics. However, if there is alcohol use or environmental exposures, the case of cancer in these individuals is higher. Another thing is I remember was one alcohol international conference somebody tried to show that there was an alcoholic with ALDH2 homozygosity. But 10 labs followed up on that and it was not true. So there is no alcoholic with ALDH2 homozygosity today.

[SLIDE CHANGE]

DR. VASILIOU: Again, this is just some of the -- you're going to find the information known as rs671. And this is again prevalent in East Asian population, but 560

million of the -- people are affected by this. And I took that from the genome database again, one we're talking for this.

Look at everything, alcohol sensitivity, alcohol dependence, susceptibility to hangover. Also, we have shown and we have included ALDH2 polymorphism. It can affect the metabolism of nitroglycerin and their susceptibility to poor response to nitroglycerin. As I said, esophageal cancer and susceptibility.

Another thing, which I'll spend a little bit later is it has been found also to be involved in what we call AMED Syndrome, which is a digenic syndrome. And I'll give you another slide to explain you that.

[SLIDE CHANGE]

DR. VASILIOU: Okay. There is a long time on changing the slides.

So instead of giving you all the epidemiological studies, I can tell you that ALDH2 it is really associated and very strong evidence with strong epidemiological studies. I don't think you're going to find any other polymorphism. So strong on a causal effect on -- you know, on a polymorphism with cancer. But this has to do with exposures. This has to do with ALDH, with the drinking of alcohol.

And you can see on the left-hand side, this is a

systematic review, a meta analysis, a modeling study, which further implicates the ALDH allele ALDH2*2. And on the right-hand side, this is just published in Science Advances, "Genetic Architecture of Alcohol Consumption Identified by Genotype-Stratified GWAS and Impact on Esophageal Cancer Risk in Japanese People." So in -- really, it says more about the role of this polymorphism that happened.

[SLIDE CHANGE]

DR. VASILIOU: So why this -- why this could be associated with cancer. Well, very simple, you have the formation of acetaldehyde, which has been classified as a Group 1 carcinogen by IARC. It is linked with multiple cancers as we said. And it forms adducts and impairs -- that's another -- the reason I'm on these slides is I want to show you that it has the ability of impairing the DNA repair mechanisms and, you know, leading to susceptibility. On the right-hand side, I have put two of the -- two nice figures from this analytical Chemical Recessive in Toxicology article, which indicates there is a metabolism. And then the metabolites can also go and cause DNA damages. And these DNA damages then they can come. They affect the DNA repair. They can affect -- they can affect, you know, also the damage.

So you may have the mutation. You may have the

damage, but as I think Fred also mentioned, there is DNA repair, which can take care of business and can correct the abnormalities and bring this normal. However, if you do have mutations in there, like BRCA1, BRCA2, then you are in trouble and we'll show you that.

[SLIDE CHANGE]

DR. VASILIOU: So very quick. This is acetaldehyde that can form with the deoxyguanosine. It can form adducts. And also, you know, you can have 2 molecules. So if aldehyde -- they can form aldehyde. They can form this methyl-gamma-hydroxy-para-dG adduct. And this is another important -- I have a couple of slides just to show you how important it is.

Remember, acetaldehyde can also induce further lipid peroxidation, which generates 400 lipid -- during lipid peroxidation, you have the formation of 400 different species -- aldehyde species. And among those aldehydes, as I told you, is malondialdehyde, 4-hydroxynonenal, acrolein, and all the other alpha, beta, and saturated aldehydes, which is very potent. And they can also induce the DNA adducts. So it's not only acetaldehyde causing the adducts, but also the lipid peroxidation.

[SLIDE CHANGE]

DR. VASILIOU: Again, this is a very nice picture

for you and for your notes -- you can see on the right hand, you have a single molecule of acetaldehyde. You have one type of adducts, 2 molecules. And also, as I said, through the reactive oxidant species, you have the formation of these adducts.

[SLIDE CHANGE]

DR. VASILIOU: And I tried to bring you as much as I could to show you that. And, of course, you can say, well, you have -- you have DNA adduct information. So what's going on?

Well, first of all, you have all the consequences of those adducts include frameshift mutations, DNA interstrand cross-links, DNA intrastrand cross-links, and you have base-pair mutations, deletions, rearrangements. And also you have double-strand break-ins, sister chromatid exchanges. So there's a lot of things that they can occur in there.

[SLIDE CHANGE]

DR. VASILIOU: This is from our review. I just put it in this morning to tell you the truth. I almost forgot about it. We have a recent review on the molecular mechanism of alcohol-induced colorectal cancer, which, for some reason, doesn't show very well because of the colors. But this picture -- I really like this, because it has the effects of ethanol on inflammation and the cytokines. And

also you can see the cytochrome P450. We have talked about the others.

Cytochrome P450 on acetaldehyde, as I said, they can still induce reactive oxygen species. They can induce lipid peroxidation. And you can have malondialdehyde, 4-hydroxynonenal. You have more adducts since going in there.

Then, of course, on the right-hand side, you have also your antioxidant systems that they can block the reactive oxidant species. But this is also another story that we could spend another 2 hours of lecture.

[SLIDE CHANGE]

DR. VASILIOU: So this is a paper that actually came in Nature. And I had so many difficulties understanding the nature of this paper, and I will explain to you why. Initially, I really liked this paper. And I will show you and I will tell you why. Sometimes you are wondering why it is.

As a principle, I love it. You know, it says that you have aldehyde, you have DNA damage. And this DNA damage if it's not repaired, this is people that they have Fanconi anemia pathways. So if the DNA is not repaired, then you can have developmental defects, fetal alcohol syndrome, bone marrow failure, and, of course, you can have cancer.

And they have done some of the experiments. They have shown that there is, you know, the combination of the ALDH2 knockout mice with Fanconi, two embryos they can have really smaller size and everything.

[SLIDE CHANGE]

DR. VASILIOU: So here is my first question on that. You can see on the left-hand side the general scheme. But I want to draw your attention to the right-hand side, which they have some cultures. And they were saying that the survival, the toxicity of acetaldehyde, it was higher on these people -- on these individuals that they had the changes on the Fanconi anemia.

Look at the levels of acetaldehyde that they have used on this. It's in the range of 8 millimolar acetaldehyde. I can assure you it's so tough to get more than 400 micromolars of acetaldehyde even in individuals with ALDH2 polymorphism that they drink alcohol. Okay. Sometimes we have to be very careful of what doses are we using, but it's -- I wrote -- I wrote actually to Nature. They didn't let me put a comment on the paper.

[SLIDE CHANGE]

DR. VASILIOU: And another thing that I was really, really, really frustrated was that -- and it was very good -- as a thought, it was very good. So BRCA2 --

if you have the effects of acetaldehyde on BRCA2, then you can -- the acetaldehyde in addition of causing adducts, it can decrease or it can attack the BRCA2 enzyme. And then you can have this induced haploinsufficiency of the enzyme, and then you can have genomic instability.

[SLIDE CHANGE]

DR. VASILIOU: And again, look at this, and I said -- what is the physiological relevance? Look at the E picture in here. And you can see this is a western blot indicating the molecular size of this protein, 250 kilodaltons in the BRCA2. And you can start seeing effects of degradation by acetaldehyde in the level of 4 and 6 millimolar. And they have used up to 30 millimolars to get complete done. I mean, I understand sometimes you have to use we're doing dioxin research. We're using high levels to identify that. But these kind of doses are kind of, you know, really unreal.

[SLIDE CHANGE]

DR. VASILIOU: Anyway, however, we have to be giving credit to the people they have identified, the BRCA1 and BRCA2, protect against endogenous aldehyde toxicity. These are very solid experiments. They have been published again.

So the whole idea, the whole story has started. If people with a BRCA2, they have -- in a combination with

the ALDH2 polymorphism, they have higher incidence. Now, this is what epidemiology can become tricky in the way of causative and association. This is a paper that was published in 2022, which says lack of the impact of ALDH2 polymorphism, the variant, on breast cancer development in Japanese with BRCA1 or 2 mutation carriers. So again, this is epidemiology. We don't have any data yet, but this is -- I always when I do my science and my lectures, I'd like to put both sides of the literature, which has been published.

[SLIDE CHANGE]

DR. VASILIOU: So this is -- this is what I was telling you before for the digenic effect. This is one. There is a combination of the alcohol dehydrogenase 5 and ALDH2 polymorphism. And this is a healthy individual. So when the enzymes both are present, you have endogenous formation of formaldehyde. Formaldehyde is capable of causing DNA damage, and then -- but the DNA repair takes care of that, so you have normal blood cells. However, if you do not have -- if you have a combination -- actually, in these individuals, what they have found for this is there is a lack of ADH5 and ALDH2, then you have decreased blood cells, okay? And if -- and this can be done by either having decreased metabolism or decreased DNA repair. So it's in both cases a very big case.

[SLIDE CHANGE]

DR. VASILIOU: One thing that I want to point out though between these two phenotypes, there is a skin hyperpigmentation. And I have to tell you we have published that in the ALDH2 knockout mice. You put them on the alcohol, there is a high skin hyperpigmentation in there, which we have shown that it increases and looks like this might be the combination for the -- for the AD -- ADED syndrome. And this is the syndrome that I told you.

[SLIDE CHANGE]

DR. VASILIOU: And this is the syndrome that I told you. It's an autosomal recessive digenic multisystem disorder characterized by global developmental delay and impaired intellectual development, onset of bone marrow failure and myelodysplastic syndrome in childhood and poor overload growth and source stretcher. So this is very well known and it's been first discovered by the Japanese group and there is a lot of studies later going on.

Anyway, the point I want to make there, one more time, anything that has to do with dysregulation of aldehyde metabolism, it has -- always have to do with something with CNS and developmental delay, intellectual development and so on.

[SLIDE CHANGE]

DR. VASILIOU: Again, I'm going to pass through this very quick. This is environmental exposures. This is endogenous source. You have cytotoxic aldehydes. You have, first of all, the aldehyde -- tier one is aldehyde detoxification system. Tier 2 is the DNA repair. If have something goes wrong, in either of those, you can have a case of the toxicity.

[SLIDE CHANGE]

DR. VASILIOU: And again, this is the same picture essentially indicating these two enzymes for this syndrome. And on the right-hand side, you can see the formaldehyde adduct, the acetaldehyde adduct, and also some interactions with protein that they occur in these individuals.

[SLIDE CHANGE]

DR. VASILIOU: So talking about vinyl acetate and DNA adducts, I found this really nice paper that they're talking and they have done LC-MS analysis. This is where the future -- this is where we're going, especially in -- I guess Fred can help me on that is the adductomics. This is an area that we're going towards to develop a more precise hazard identification and risk assessment is identifying this and this adducts that they may occur. This is a model though of having rats that they expose them to vinyl acetate, inhalation, and they identify those

adducts into the nasal cavity and to a very less extent into the systemic circulation, which means whatever is there, it's there to stay. And it may cause problems. And essentially, vinyl acetate I think is associated with some increased distance with nasal cancers.

[SLIDE CHANGE]

DR. VASILIOU: And this is just the basic reaction. Vinyl acetate, through our carboxylesterase, it's converted again to acetaldehyde. And, of course, it can form the adducts. These adducts have been measured and identified. And remember, the other thing is you can also have an increased lipid peroxidation, which these people did not include there. But this is the adductomics in here. And these adducts were identified in the nasal cavity of this.

[SLIDE CHANGE]

DR. VASILIOU: This was a duplicated slide.

I apologize for that.

[SLIDE CHANGE]

DR. VASILIOU: Again, what I want to tell you there is DNA repair pathways that they protect against not only acetaldehyde but aldehyde mutagenesis. And this is a beautiful paper I just found that was published in January 8, 2024. It's still BioRxiv. I said published, but essentially, they have used the yeast and they have found

multiple pathways by which these adducts they can be prevented. So the importance of the DNA repair is huge. And any changes, any polymorphisms there or any effects that you have, and this is -- we know that there are several in human population, that can make the individuals more susceptible to those environmental chemicals.

[SLIDE CHANGE]

DR. VASILIOU: Another thing that I want to stress out and I'm getting close to be done is the importance of the endogenous oxidative stress. I remember Bruce Ames they were saying there are about 10 to the 8 oxidative hits in our DNA per minute. Okay. So what happens in that, what happens? Well, you have the oxidative stress and you can have that. So the whole idea is how you can distinguish the endogenous and exogenous. How you can take risk assessment to the next level and how you can do a total global thing, because it could be endogenous, it could be exogenous, but they both could be interlinked.

So in this method, this -- these people, this group developed again an LC-MS/MS method, which essentially use stable isotopes onto the exogenous molecule. And then they can identify the adducts by that. I think this is huge. This is really important. And this will kind of open the field of not only going further deep

into the mechanism, but also help us to determine a little bit of the better risk assessment.

[SLIDE CHANGE]

DR. VASILIOU: So I know I got you tired, but I want to tell you that the ALDH2 polymorphism is associated with increased cancer incidence following the exposures to environmental chemicals. The mechanism of induced DNA adduct formation and decreased repair mechanism. There is an increased risk for individuals with ALDH2 polymorphism, particularly when coupled with conditions of impaired DNA repair, such as Fanconi anemia. Things to consider that I did not have the time to go over today is the effects of aldehydes and other aldehyde -- of acetaldehydes and other aldehydes, you know, onto the epigenome, and also something that I have mentioned about 30 years ago. I never got into it, how acetaldehyde could affect mitochondrial DNA?

And the reason is ALDH2 is a mitochondrial enzyme, so acetaldehyde does go to the mitochondria. So this is something that, you know, perhaps we need to think about it and we need to, you know, get back into it.

[SLIDE CHANGE]

DR. VASILIOU: So thank you very much for your attention.

(Applause).

CHAIR LOOMIS: Thank you very much, Dr. Vasiliou. I think we have a few minutes again for questions of clarifications, if there are any?

This way. That way. Yes.

COMMITTEE MEMBER BESARATINIA: Thank you very much, Doctor. It's on. In one of your introductory slides, you mentioned that ALDH has antioxidant properties as well as absorbs UV. In its capacity to absorb UV, does it function like a chromophore and then undergo photosynthesization reaction to produce ROS and cause oxidative damage as well or does it do it through a different mechanism?

DR. VASILIOU: Well, that's a good question. The question is how the U -- how ALDH can absorb UV. This is based on our study and this is a beautiful. Our corneal -- our cornea in the mice, they express aldehyde dehydrogenase 3A1 as much as about 30 percent of the total water soluble protein. And this is how I started looking at the effects of that -- how -- why it's there. I mean, Joram Piatigorsky has called that as gene sharing. So the lens -- essentially, this started from the lens and we extend it into the cornea. The lens, they're protein-containing organs that they have taken several genes. And they essentially use them for having the transparency.

So we believe that they did the same thing with aldehyde dehydrogenase. Anyway, to make a long story short, we have done several studies that they have shown that UV, through some amino acid. I don't remember which one it is, they absorb UVA, and they commit suicide. So what they do is they absorb the UVA or the other reactive oxygen species, and they protect further oxidative stress in delicate tissues like cornea.

And I don't think it's the case that they can further -- they can have further induction. It's on the protective side. So they -- just like the -- some of the DNA repair enzymes would they get them -- the methylation and they commit suicide and it's the same exactly thing.

COMMITTEE MEMBER BESARATINIA: Thank you.

DR. VASILIOU: And we have also shown an indirect antioxidant capacity by which ALDH helps in the regeneration of the NADPH, which converts GSSG to GSH, and you have further glutathione to respond.

CHAIR LOOMIS: Okay. Thanks. Are there any other questions of clarification here?

No. No. All right. Well, thank you very much. Appreciate it.

DR. VASILIOU: Thank you.

CHAIR LOOMIS: At this point, I'm going to propose we break for lunch. And before we do that, I'm

supposed to read you a reminder about the Bagley-Keene Open Meeting Law.

So during lunch breaks, you're not allowed to talk amongst yourselves about the subject matter of the meeting. That includes phone calls, texts, chat, and in-person discussion. It's best if you don't talk to third-parties about the items being discussed. And if you do, then you need to disclose that fact that you had a discussion and give the general content of the discussion, so it's part of the public record.

So it's recommended that you talk about something else, like, you know, the weather or whatever. All right. So lunch is scheduled for 45 minutes. It's now 12:16, so let's convene back here at 1 o'clock.

COMMITTEE MEMBER EASTMOND: Can I ask a question to your attorney. Is that really necessary not to talk about the subject of the meeting with the speakers during lunch.

SENIOR ATTORNEY MORIOKA: The goal is that all discussions about the relevant materials are in front of the public, so that if you have pertinent discussions, then the public has knowledge about what you're talking about. But I understand that there are no vote -- that there is no voting at this particular meeting, so it's not as though you're influencing a vote in any way. We just

prefer that you have discussions about the meeting materials in a public setting.

COMMITTEE MEMBER EASTMOND: Okay.

CHAIR LOOMIS: Okay. Okay. Let's reconvene at 1 o'clock. And lunch is served somewhere.

(Off record: 12:17 p.m.)

(Thereupon a lunch break was taken.)

(On record: 1:03 p.m.)

CHAIR LOOMIS: Can the committee reconvene, please.

Very good. I hope everyone had a satisfying lunch. Good break. The next item on the agenda is public comment opportunity. So the public may comment on any aspect of the presentations that we've heard this morning.

I think the instructions for public comment are about to be shown on the screen.

(Thereupon a slide presentation).

CHAIR LOOMIS: There they are. So as a reminder, individuals who are in person and want to make an oral comment have been asked to fill out a blue comment card located in the back of the room. We'll call those present in person to provide their comments. We ask you to approach the microphone and state your name and affiliation before making your comment.

Anyone joining by Zoom who wants to make an oral

comment can do that by raising their hand in Zoom. There's an icon for that for those not familiar with it. When you raise your hand, your name will be called and you'll be prompted to unmute yourself. Please do that. State your name, and affiliation, and provide your comment. Public comments, whether in person or via Zoom will be limited to 5 minutes.

So at this point, are there any comment cards? I haven't been given any yet.

Apparently, no. So very good.

There seem to be no comments in the room. Are there any participants on Zoom who wish to make a comment?

Okay. We see none. So it appears there are no public comments on this morning's items.

And so then we'll move on to discussion of the presentations with the Committee and speakers. And I see we have all the speakers here and most of the Committee. So this is an opportunity for the Committee and speakers to interact and discuss the material that has been presented this morning.

So I first invite members of the Committee to ask questions or comment on what we've heard?

Anything?

Well, I have a question or a comment. I'm not sure which it is. But the material we were offered this

morning was particularly interesting and, you know, one of the questions that comes up for me in looking over this information is that there is kind of a paradox about the relative risk measure of association, which is that relative risks tend to be higher in association with kind of the rare characteristics, not so high an association with more common characteristics. We could see that on Dr. Ginsberg's slides, for example.

And so, you know, as a clinician, I might be worried about high relative risk, but as a public health official, I might be more worried about the common characteristics, even if they have lower relative risks. However, there's another twist here, which is that those high risk individuals may be individuals that for purposes which -- with which this Committee is concerned, we would want to create a more protective standard. So I would be interested in the speaker's thoughts on that dilemma that we face about how to use the type of information they've all presented.

DR. GINSBERG: I think I'm on. So I'll take a first crack at that. That was my last slide, which I presented a little bit of a framework for thinking about it. And, you know, for example, if you are at a hundred-fold, if we can calculate a scenario through multiple polymorphisms where someone, if they had the bad

deck of cards so to speak, and three or four susceptibility genes in the same person, exists at least at 0.1 percent of the population in there. And you can calculate that that's a hundred-fold higher risk, that that might be a scenario where we'd want to consider at least separately evaluating, rather than trying to blend that tail of the curve of the susceptibility curve into some overall population approach.

So the question, it's really a policy call, just like one in a million de minimis risk was a policy call back in the 1950s. You know, what is the size of the population, and the excessive risk, and the certainty that we have around that, that presents enough of a subpopulation concern to treat them separately.

CHAIR LOOMIS: Thanks. Very good.

Other questions, comments from the Committee?

COMMITTEE MEMBER EASTMOND: Sorry, I came in late. I'm not -- are you just having general follow-up questions to the entire group?

CHAIR LOOMIS: Yes. This is an opportunity for the Committee and the speakers to interact about the subject matter.

COMMITTEE MEMBER EASTMOND: Well, this is a related question. So you have variations in enzyme levels that are caused by genetic polymorphisms. And like Fred

and others mentioned, you also have enzyme inductions so you can get dramatic differences in enzyme levels caused by factors which would be environmental factors.

And I don't -- I mean, I think the question as OEHHA thinks about this is how do you integrate these together, because not only do you have the genetic polymorphisms, but you have phenotypic variants. And this is -- and sometimes it may be predictable, such talked about alcohol consumption or alcoholics, but in other cases is not very predictable. And that enzyme induction may be affecting a specific subpopulation as well.

So I find this to be challenging. I appreciate Gary's thoughts on how you might fold this in, but I see that as another subpopulation I might worry about or those who were prone to enzyme induction.

DR. GUENGERICH: Yeah. Maybe I could -- I don't know if I can bring any clarity. Maybe probably some more confusion to this. But again, Dan Nebert again has written a lot about this. And he's written a number of articles over the years about the influence of enzyme induction. Now, maybe even from my talk, you got the idea that inducing AHH and the AH receptor was bad, but Dan has basically written a lot about this. And in many cases, it's actually good and it's actually very protective from injury. So it's not like there's one phenomenon that it's

always good and -- or always bad. And in fact, it goes back to a classic, something called the Richardson Experiment in 1952. When giving small amounts of one carcinogen would actually protect rats from another carcinogen. And now, we understand that was due to enzyme induction, so it's complicated.

And some of this stuff I covered in my own class lectures, but some of it Dan Nebert has also written about as well. And it can depend on where you're exposed. And he talks about proximal and distal targets. So basically there's a difference in terms of whether the environmental chemical -- we'll just call it the environmental chemical is going to hit a target that's -- where the enzymes are or where -- you know, it depends where everything is in the body too. So things can be protective or they can actually lead to more destruction. So it gets -- it gets very complicated in a hurry that is, I guess, my bottom line.

And sometimes I wonder if we'll ever really understand. And maybe artificial intelligence will eventually solve everything. But I'm not sure we're quite there yet. I do -- you know, I mean, I'm in -- I guess, we throw around a lot of these extra factors, I mean, you know, in terms of risk assessment. You know, a 10 for -- a 10 for -- or maybe more for comparing animals to people.

And then another factor for comparing people, but most of the time we don't really know if these are real, or we're just sort of trying to be more protective, or if it's really doing anything or not.

DR. GINSBERG: Fred, that applies to non-carcinogenic risk assessment. But for carcinogens, there is none of these uncertainty factors. It's just based upon human equivalent dose from animal evidence, but applying the animal based -- unless it's an occupational study. So for cancer, we don't use an uncertainty factor, which is part of the point of maybe thinking about polymorphisms as -- and we did in Science and Decisions in 2009, there was some proposals along these lines to bring some of this variability and to more overtly bring variability into human cancer risk assessment.

DR. GUENGERICH: Yeah, I guess I would be concerned though. Again, I've -- you know, there are a number of qualifications about animal models. There are types of cancer we see that are specific to rodents. I think that's generally agreed on. And we don't really -- we don't have -- I don't think we have a great database on that in terms of the extrapolation frankly speaking.

DR. VASILIOU: Actually, well, a couple of things and I want to go back with what Fred says. If it's a simple compound. You mentioned alcohol. Yes, the

alcohol -- and you don't have to be alcoholic. You just have to drink alcohol every day and you have increased levels of cytochrome P450 2E1, that already makes you more prone to certain other exposures. Okay. And it doesn't have to be alcohol. It could be over-the-counter medications. It could be starvation. Okay.

The other thing I want to tell you is -- and this was something that mentioned again by Fred, the acetaminophen in this particular case why this is important. Actually, there is a syndrome, which has been established. It's called acetaminophen ethanol syndrome. So if you have the two you're on already, you're guarantee you're going to have, you know, toxicity or some other consequences.

The point is when you go to simple models and simple questions, you can get that. Now to make life complicated, it's not only alcohol, it's not only over-the-counter, we'll discuss tomorrow we have the case 1,4-dioxane. We found first time 1,4-dioxane induced Cytochrome P450 2E1. So you can have -- you don't need to drink alcohol. You don't need to take over-the-counter, you just drink your water and if your well is contaminated by 1,4-dioxane, it doesn't have to be high levels. It will induce in the long term your 2E1. So that's one thing.

The other thing is the second point I want to make is we are exposed to low levels of chemical mixtures and how you can get really the effects from that, Fred gave you a hint. We need to develop the algorithms. We need to develop the artificial intelligence that we can take into account, not only the multiple exposures, but also genetic background. We just published a paper on 1,400 phytochemicals present in the olive oil and how this can interact with Alzheimer's pathway on protein DNA interactions and how -- because there is substantial evidence that olive oil, for example, it could prevent or it can work against Alzheimer's disease.

In that study, that algorithm worked very well. So we ended up with 10 chemicals, which actually there is some substantial evidence. And also, Fred said algorithms are good. They're going to generate models. We need to validate. So it's a long way to get straight answers from that, unless you have classic examples, such as the 2E1 or, you know, benzo(a)pyrene.

And another phenomenon that I would like to bring to your attention, Fred also mentioned that a little bit, is what we called hormesis. Hormesis is when you're exposed to certain oxidants or certain conditions your genes are upregulated and they can make you more strong. We have one case that we have mice with low levels of

glutathione. And I thought we were going to develop the best ever model for liver toxicity. We subjected these mice to alcohol and these mice have better health even when you fill them with alcohol. There is no effect.

Why? Because the endogenous oxidative stress generated by low levels of glutathione induces this hormetic response, which in turn turns on the regulator of your metabolism, which is AMPK first regulates that, and makes these mice almost -- you know, they're resistant to ozone toxicity, they're resistant to certain conditions.

So another thing that life can be complicated is by exposure. In some of these levels -- you know, you get -- you don't have to have toxicity. So this particular model, we don't eliminate completely glutathione. We eliminate it to the point that we'll elicit an hormetic response. If we wipe out completely glutathione, either there is no life or in every tissue we're doing, we have different effects.

CHAIR LOOMIS: Very good. Other question?

Down here.

COMMITTEE MEMBER WANG: So I guess I'm hearing the proverbial more data are necessary. And I'm curious. I guess I would like to hear from each of the three presenters is -- what would they consider -- is there anything that you've presented that you believe is

actionable today?

DR. GINSBERG: Yeah. From a risk assessment perspective, I'll -- I know this is being recorded, but I want to be careful in not raising expectations too far. But where we have highly influential polymorphisms like a null polymorphism in a well-defined critical detoxification pathway that you can follow around in populations and you have the epidemiological evidence that it does translate to risk, not just, you know, in a cell -- in vitro cell culture system where you can, you know, isolate this polymorphism and show more DNA adducts or something. But when you actually see it in populations, that must mean that it's fairly influential, fairly penetrant and that's worth thinking about from a risk assessment perspective.

So, you know, we talked about a couple of those today, aldehyde dehydrogenase 2 when your polymorphic, in that you have no function and the result of that is, you know, some of the increases Dr. Vasiliou talked about. I mean, that's something that might be actionable.

Glutathione transferases when you have multiple ones that are knocked out, you know, you're leaving the population more at risk for oxidant stress and things that glutathione normally will help take care of. NQO1 knockout and bone marrow toxicity. So, you know, I think

there's a couple of examples and I think if -- from a regulatory perspective, if people focused on the low-hanging fruit and what are some of the clearest ones to try to create policy around, that would be a starting point and then work from there.

CHAIR LOOMIS: Other questions, comments from the Committee?

DR. GINSBERG: I think that was a question for all three of us.

CHAIR LOOMIS: Oh, right. Yes. Sorry, yeah, it was a question for all three. I apologize. Go ahead.

DR. GUENGERICH: Well, I'll take the next stab. I actually -- I think I agree with your point about the nulls. I think the problem we have is that as indicated this morning, there are so many variants out there in the population that in terms of characterizing exactly what the effects of each of those are is going to take a long, long time and you pretty much will have to do that in vitro at least at first.

The nulls, now they're gone. I was going to say the other problem with the variants if they're in the coding region, they actually may have different effects depending on what the substrate for the enzyme is. This is well known in drug metabolism for instance. So the nulls, there you actually do lose the gene and it -- in a

way, it's easier.

Having said that, people, you know, they've kicked around the glutathione transferase polymorphisms for a while. And those are real deletions. And we've got some results, but a lot of things still aren't clear too. So I think that's probably one useful thing to do.

I was going to say the other thing Dr. Vasilis brought up is this matter of hormesis. I didn't talk about that in my talk. I talk about it in actually the first lecture in my toxicology course. And that -- again, the concept that a little bit of something -- a little bit of damage is good for you, and there are actually very good biochemical reasons for this now in terms of the Nrf2 system and things like that.

So the problem is that I'm sure drives regulators crazy, that a little bit of something is actually -- you know, is good for you and, you know more of its bad, because how do you actually regulate things when you're down at a very low level, because they may be protecting you from other things. Another example is metallothionein. Basically, a little bit of toxic metal is good for you because it induces metallothionein and which will protect you from a big overload.

So we have a bunch of problems. And like I say, the nulls is not a bad way to consider things. I think

though ultimately -- I don't think -- I guess I would make the point and maybe I tried to do that this morning, that I think we're going to be on shaky grounds if we go with only epidemiology and don't have mechanisms to go along with it, and some kind of viable mechanism, and some kind of system. And I'll turn it over.

DR. VASILIOU: And this is what I call translational epidemiology. So you have your epidemiological study. And then you have the model, either it could be an animal model or it could be a tissue on a chip, or a tissue, a 3D culture, that your epidemiology study shows an association. Then you go and prove that this is the mechanism. I think that's where we need to develop healthy regulations see if the epidemiological studies are really supported by mechanistic studies to prove what -- otherwise, it's just an association, confounders could be millions, genes could be many.

Another thing that although it does sound like a science fiction that I think the deep learning and those algorithms will be very soon helping us in determining these kind of factors without decreasing the research -- the basic research we're doing, but combining all the information, and especially another thing now is that we can really substantiate the role of the epidemiology. You

have a lot of clinical records, medical records that are available now that you can combine all those in your epidemiological study. So as long as you have a mechanism to support that, that's what essentially what it is.

Thank you.

CHAIR LOOMIS: Thank you. I think Dr. Bush had a comment or question.

COMMITTEE MEMBER BUSH: Not specific, but I'll ask since I've got the microphone now.

And I guess this goes more to Dr. Vasiliou and Dr. Guengerich, where are we in getting a catalog of these polymorphisms of these -- you know, the phase 1, phase 2 enzymes, but at a cell type specific level, because that could be instructive for us. For example, if there is a known chemical that happens to be, you know, prevalent or causing something related to a squamous cell or some other kind of epithelial related cancer, I mean, do we have that information yet? So I implore you, if we don't, can we get that?

DR. GUENGERICH: Yeah. Actually, you know, it's not perfect, but there's some called the protein atlas, which is online and it's pretty good. It's not perfect, but basically that will tell you every tissue and cell, you know, what the levels of the RNA for a particular gene are, and the protein -- not always the protein, of them.

But yeah, that's not bad.

Now, it won't tell you anything about the variants and the polymorphisms though. But we know where things are expressed by and large. The -- I mean, it's not perfect, because if you look at that, sometimes there's something we call the -- sometimes the grass, that is there will be minor levels in all kinds of tissues, but it's not bad to a first approximation. One thing I would like to comment on though, I guess one of the problems -- and I think I mentioned it first about artificial intelligence. One of the problems is, potentially these AI machines are very good at gathering stuff that's out there. But if they gather up the junk, you will get junk out of them. And so I don't -- and I'm not a computer scientist, so I don't know how to solve that problem. So we have to be a little -- I think we still have to be a little careful. But, yeah, we do know quite a bit about where these things are expressed.

DR. VASILIOU: And I'll correct a little bit Fred about that. Yes, the AI gets a lot. It gets everything that can read. You know, it's a computer. However, that's where the human factor is that we're going to curate, you know, the particular studies and -- is what we call train the algorithm. Okay. And train the algorithm is providing the substantial base. And, of course, there

are some junk studies. That's why I'm saying everything that can be found by artificial intelligence they still have to be -- you know, a human factor have to be there. Look, artificial intelligence right now, it helps to do -- diagnosis -- clinical diagnosis. You can do histology by -- you can do face recognition for alcohol-induced fetal syndrome. You know, you can get that and you can tell if it is or not.

The point is still you need a doctor when you have the prog -- when you have the diagnosis when the artificial intelligence does, just in case that something happens. So we're not there yet that the computers will completely substitute us, but we need to work together. We need to take advance of that.

For example, what you mention is very important. I want to make life a little bit more complicated though, because you can have this protein gene express -- the -- I'm sorry, the gene expression tissue cell, which a beautiful database. You know, you can get it -- now, the polymorphism occurs in your DNA, so it will be everywhere.

The point is if the metabolism occurs, mostly in the liver and then as the metabolites would say are saving the tissue, that's another case. So there are quite a few things. And believe me, you can put all those factors into the artificial intelligence. I'm a big believer in

artificial intelligence with all the carefulness that we need to pay on that, as Dr. Guengerich said.

DR. GINSBERG: And I'll just add one more complicating factor which has been mentioned today, which is the methylation patterns and how genes are regulated outside of the things that we've been talking about so far. So that -- on top of all the genetic -- genotype changes, genotype effect on phenotype is one thing, but effects on phenotype that have to do with how other gene regulatory mechanisms will also affect, especially the upstream polymorphisms that are affecting promoter regions, because you have so many things affecting how much expression there's going to be in a certain gene. But where you have a null -- again, I'll speak up for the null polymorphisms, it doesn't matter, you know, what's going on in the regulatory sequence. If it's just a defective version of the gene that's inheritable, that would be more likely to be penetrant.

CHAIR LOOMIS: Okay. Thank you. I think we've taken more time than we had scheduled for this item, but perhaps we'll just quickly see if there are any burning questions or comments from the Committee before we move on.

COMMITTEE MEMBER EASTMOND: I have one, unless you're really short on time.

CHAIR LOOMIS: Really short?

COMMITTEE MEMBER EASTMOND: Yes.

CHAIR LOOMIS: Well, you can go ahead, if it's -- if it's quick.

COMMITTEE MEMBER EASTMOND: I'll just say that for me one of the most fascinating stories on the aldehyde dehydrogenase 2 story is that the homozygotes sort of wild type and the homozygotes who are the *2 have inefficient enzyme activity, they're basically at very low risk of esophageal cancer. It's the heterozygotes. And so because they -- homozygotes that are recessive basically, they can't tolerate alcohol, so they don't drink it. So they're at low risk of cancer. It's the heterozygotes who have less efficient aldehyde dehydrogenase 2 activity. They consume more alcohol, and they're at higher risk, and so they're the ones that show up with the cancer.

So if you look at this from a sort of purely biochemical point of view, you might miss that because you have to superimpose the behavioral aspects of what happened in addition to sort of mechanistic studies that you think of the genetics. So for me, that's one of the most fascinating aspects of that story. Sorry.

CHAIR LOOMIS: Good. Thanks.

At this point, we should move on. We do have a break scheduled after this item, but I'm going to propose

that since we just came back from lunch, we'll postpone that for a bit and see if we need it later.

And so that allows us to move on to the second agenda item, which concerns committee input on staff proposal to streamline several sections of the cancer hazard identification documents. And I believe that Dr. Sun is going to present that.

(Thereupon a slide presentation).

DR. SUN: Hello. Good afternoon. I will give a brief overview of OEHHA's proposal to streamline three sections of our cancer hazard identification documents or HIDs. The HID is provided to the CIC for their deliberation in determining whether a chemical should be identified as a carcinogen under Proposition 65. Other materials also provided for the Committee's deliberation include all the references and the public comments received. The focus of today's proposal is on the HID.

[SLIDE CHANGE]

DR. SUN: Here is an outline of my presentation today. I'll first provide an overview of the goals of the proposal and today's discussion with the Committee and then talk about specific changes we're proposing to the following sections of the HIDs: the introduction, carcinogenicity studies in humans, and carcinogenicity studies in animals.

[SLIDE CHANGE]

DR. SUN: The goal of this proposal is to streamline three sections of the HID by focusing on the most informative studies and limiting the scope of discussion for the less informative data. And the goal for today's discussion is to request the CIC's input on the proposal.

[SLIDE CHANGE]

DR. SUN: I'll give a brief introduction to the structure of the proposal.

For the introduction section of the HID, which is relatively less complex than the other sections, the proposal presents the changes and examples of how these changes would be implemented.

The carcinogenicity studies in humans and animal sections are the key elements of the HID, and the proposal for each of these two sections include discussion of general considerations on study informativeness, the proposed changes, the proposed organization, and examples of how these proposed changes would be implemented, using text from the 2022 bisphenol A or BPA HID. While these examples are helpful to show how certain sections would look like with the changes, they reflect the specific database available for BPA.

What constitutes most informative may vary by

chemical, and we will need to retain some flexibility to adapt to each specific assessment in the future. I also want to note that the BPA HID is only being used as an example, and we are not proposing any actual changes to that HID.

[SLIDE CHANGE]

DR. SUN: The introduction section of the HID includes chemical identity and properties, exposure-related information, and reviews by other health agencies. As the HID focuses on identifying hazard, we propose to shorten the description of exposure-related information by providing a more concise summary of production, sources and uses, and occurrence and exposure. We are not proposing any changes in the chemical identity and reviews by other health agencies sections.

The section on production, sources and uses would be limited to 1 to 2 paragraphs. It would briefly summarize information on the production of the chemical, such as volume of production. It would broadly indicate sources of exposure, and common uses of the chemical that may lead to potential human exposure, for example uses in consumer products.

For occurrence and exposure, this section would be limited to 1 to 2 paragraphs. It would briefly summarize the occurrence of the chemical in different

environmental media, for example air or water, and human biomonitoring findings, for example in blood or urine samples with a focus on California. Magnitude or temporal trend of exposure may be briefly discussed.

[SLIDE CHANGE]

DR. SUN: Here are the proposed changes for the carcinogenicity studies in humans section. This part of the HID begins with a section on key issues in the consideration of available studies before going into the presentation of studies by cancer site or type. As was done for the recent HIDs on PFOS and BPA, the key issues section highlights topics relevant to the available database, such as exposure assessment limitations, study design limitations, confounding and other biases.

Therefore, readers will be familiar with these issues when they read summaries of specific studies. You can see that proposed changes are based on how informative the studies are. I will discuss details on such considerations on the next slide. For the most informative studies, there will be no change. They will continue to be summarized in the text and in tables.

For less informative studies, issues contributing to that determination will be discussed and the studies briefly summarized.

Studies of very limited informativeness will be

mentioned in the text with issues contributing to that determination and included as a bibliography list provided in an appendix. For those individual cancer sites or types where data are very limited, the available studies will be mentioned in the text and provided in the bibliography.

[SLIDE CHANGE]

DR. SUN: I'd like to first note that the factors that define an informative epidemiologic study may differ by the chemical and exposure-outcome pair. The factors listed on this slide are for general considerations. And secondly, for each chemical evaluated, we will be faced with a different database of available studies and different key issues.

Regarding study design, generally, among observational epidemiologic studies, a greater focus is given to cohort and case-control studies. Studies of cross-sectional design are often less informative for hazard identification, as they measure exposure and outcome at the same time. Similarly, descriptive studies are often less informative, but there are examples where ecologic studies and case-series, respectively, have provided crucial evidence, as in the cancer classifications of arsenic and aristolochic acid by IARC.

Besides study design, there are factors specific

to each study that can also affect the sensitivity and ability to detect a true association between the exposure and the outcome. On this slide is shown a non-exhaustive list of potential biases to be considered in such evaluations. Other factors that can impact study informativeness include sample size, whether there is adequate exposure contrast, and whether there is sufficient follow-up to detect the presence of cancer.

An example of a study we would treat as non-informative would be a study of a chemical with a very short half life, on the scale of hours, in a population with infrequent exposures, where study participant exposures were categorized based on a single spot urine sample.

Another example of a study we would treat as non-informative would be a cross-sectional study of exposure to a chemical with a short half-life, in a population expected to have variable patterns of exposure over time, and for a cancer outcome generally associated with a long time to develop.

For these examples, these studies would be included in a list in appendix, but would not be discussed at any length in the HID.

[SLIDE CHANGE]

DR. SUN: This slide shows the proposed changes

to the carcinogenicity studies in animals section. Similar to the epidemiologic studies section, the most informative animal studies will continue to be discussed as they are now in the text and in tables. I will go over the considerations of informativeness of these studies on the next slide. The less informative studies will be briefly summarized without detailed description and without tables. The least informative studies with study designs and other features that result in considerable uncertainty in attributing the tumorigenic outcome to a specific chemical exposure will be mentioned and listed in the bibliography.

[SLIDE CHANGE]

DR. SUN: Similar to the considerations for informativeness for epidemiologic studies, the informativeness of animal studies is also determined by study design and other factors.

Regarding different study designs, long-term carcinogenicity studies, also known as animal cancer bioassays, involving chronic exposure for most of the lifespan of an animal are generally accepted as scientifically valid testing methods for evaluation of chemical carcinogenicity. We generally consider animal cancer bioassays as the most informative. No changes are being proposed to the way these studies will be presented.

Exceptionally, subchronic or short-term studies, when they are adequately designed and conducted, will be reported when there is evidence that the carcinogenicity of the chemical has a short latency period.

Other, generally less informative, animal studies for purposes of cancer hazard identification include studies using genetically engineered animal models, other types of model systems using normal cells, and tumor initiation-promotion studies. These less informative studies will be briefly summarized without detailed descriptions or tables.

Co-carcinogenicity studies and xenograft studies using cancer cells, which are generally considered the least informative of studies, will be mentioned and listed in the bibliography. While these less and least informative studies may shed light on potential mechanisms of action, their contributions to the determination of carcinogenicity rest on the overall consistency of evidence.

Although not shown on the slide, specific factors may also affect the informativeness of the study. These include route, doses, controls, and others. For example, the pharmacokinetics of the chemical may play a key role in assessing study quality.

[SLIDE CHANGE]

DR. SUN: That's my last slide. Thank you.

We're happy to answer any clarifying questions from the Committee.

CHAIR LOOMIS: So this opportunity is for clarifying questions and then we'll move on to a more general discussion, so questions about details on...

COMMITTEE MEMBER EASTMOND: This could fit in both categories, but Meng, thanks. I've just been thinking, I'm wondering who's the target audience for this document? Is it the Committee, or is it the public in general, or both?

DR. SUN: I would say it's the Committee. We develop the document for you to use to evaluate the carcinogenicity of the chemical.

COMMITTEE MEMBER EASTMOND: Okay.

CHAIR LOOMIS: Other clarifying -- yep.

COMMITTEE MEMBER LANDOLPH: I've been very happy with the HIDs. They usually give me what I need to know in a timely manner and with sufficient detail. So I don't want you to lock yourself into a corner too much. Don't make the rules so rigid that you can't change your mind and change the standards a little bit maybe for a unique chemical or something like that. So I don't think you have to make phase changes, you know, real huge changes. If you want to shorten them a little bit, that's fine with

me.

And conversely, if occasionally you feel you have to add something to bring it to the attention of our Committee, I think that's fine too. So I, in general, agree with the thrust of this effort and I think your group has always done a very good job. So don't feel like you have to remake everything. Slight tweaks would be fine.

CHAIR LOOMIS: Other questions of clarification before we move to general discussion?

COMMITTEE MEMBER BESARATINIA: We'll come back and discuss this?

CHAIR LOOMIS: Yeah, we'll discuss the substance in a moment, but I think this is just to, you know, kind of get the facts straight.

No more.

CHAIR LOOMIS: Yeah, Martha.

DR. SANDY: Yes. So if I could just comment that one of the reasons we're bringing this proposal to you is we're -- we do develop the document for your use, so we want to get feedback from you. And the last document you saw was quite large. The were many, many studies. And we're looking to get some feedback. If you can say, oh, yeah, some of those that were in the appendix maybe -- you know, you keep doing the way -- what you did or maybe a

list, a bibliography list of those studies is enough. We're just throwing some ideas out and looking for your input.

CHAIR LOOMIS: Yeah. Thanks, Martha. Just reiterate that I know the staff are looking for some feedback on how to improve the HIDs, if that's something that would be helpful. But, you know, if the Committee likes them just the way they are, that's also okay.

I'll go ahead and make a quick comment, since as we segue into the second part of the discussion here, I like the proposed changes actually. Perhaps this is a bit more work for the staff to try to segregate studies by informativeness that will work upfront, but it creates a more streamlined document that's easier to read. And I think it's consistent with trends in a lot of other agencies. When I was with IARC we moved in a similar direction. And I think our reading public and users of the monographs found that to be quite helpful. So I support the move to streamline somewhat the HIDs.

And I'll invite comments from the rest of the Committee.

COMMITTEE MEMBER EASTMOND: I support the idea to streamline the documents as well. I'm hoping that in the streamlined, things like the exposure assessment, there will be enough references or information that someone

could chase down the actual information. But I trust you'll do that. But I'm all in favor of making life easier for us and for you, if we can do it.

COMMITTEE MEMBER STERN: Thank you. I definitely like the idea of streamlining the document, because I think it's something that we organically do when we are reviewing the literature anyway, putting studies in piles. So if that helps the work that you guys do in kind of predigesting that, that would be helpful to us. The only comment I have is for the human epidemiological studies. I think it's not as straightforward perhaps as the animal studies where you can put a priori some guidelines of these are studies that we think are informative and these are not, so it's easier to put them in different piles.

I think with the human literature is very -- as it was mentioned, it's very dependent on what has been done, what's available. You know, we all know what the idea of a study might look like, but nobody may have done that study yet. So sometimes we are stuck with what's available and then we have to kind of streamline from that. So I think it's going to be hard to put criteria, a priori of what is going to be, you know, there's obvious studies, like studies that show -- that report no associations for an exposure obviously are not informative, so we shouldn't even have to look at those.

But with these studies, for example, with the BPA example, the two studies that use a cohort, within the universe of available studies, those studies that are from a cohort where the exposure was mentioned before the outcome developed, you know, that's as good as it gets. True, it was one measurement. That's not informative, but there are no other studies that have done anything different. So I think if we went with this approach, where we consider them non-informative, we wouldn't be able to say anything about what the evidence is telling us. I think -- so I think my proposal moving forward would be perhaps before the documents are done, we could have a conversation based on that particular agent, and based on what we know the evidence looks like and kind of decide organically this is what's available.

Within these studies, this is what we're going to consider informative, and this is what is not going to be considered informative, because I think it's going to change with each agent that we evaluate and it's going to be hard to put a hard criteria. I think we may end up -- we may end up in situations where we have nothing to discuss if we put like a harsh criteria a priori.

And I think that's -- Dr. Loomis was saying previously, I think organically other agencies have been moving in that direction, but I feel like it's something

where the committee has to be involved in making those decisions, because otherwise we may end up with nothing to discuss on the table.

CHAIR LOOMIS: Perhaps I'll follow up on that comment really quickly. I see there are others, but you know one thing that you see in some other schematics for evaluating human epidemiologic evidence is a sort of hierarchy of studies. I don't see that here -- study designs, that is. I don't see that here and I'm very happy that it's not here, because I think that's kind of a trap, right, that all observational studies get relegated to some second or third level of evidence. So I think we want to avoid that.

And now I see that there are others who wish to comment.

Dr. McDonald, why don't you go first.

COMMITTEE MEMBER McDONALD: Yes. Thank you. I also generally am quite favorable with the proposals to streamline. With respect to the animal carcinogenicity section, I always think it's a very good job you guys do. It's a good level of detail. Focus on the strengths and focus on limitations as well. I think we've said in other meetings, we really like the format of the data tables as they are that I've -- they're very easy to look at and get right to the point.

I'm favorable with de-emphasizing those other studies, like tumor promotion in non-mammalian species, co-cancer. But I really want you to retain that professional judgment and flexibility. If you say -- see a knockout mouse study, for example, that really helps tell a story mechanistically, bring it forward and don't just keep it in a line item in a table. So, you know, just use your flexibility and your judgment.

COMMITTEE MEMBER WANG: Yeah, I just wanted to second what Dr. Stern commented on. I think in looking at the example that you provided on prostate cancer, I think there can be a middle ground, right? I think there's some Simplifying that can be done, perhaps with the text, the paragraph beforehand. But me personally, the tables, you know, is very informative and I think that my interpretation of the studies would have been very different with that table versus the condensed version. I think with the condensed version as Dr. Stern said, I think there wouldn't be much to discuss at all. So I would second the simplifying, but maybe striking a compromise.

CHAIR LOOMIS: Thanks. Other input. Dr. Bush.

COMMITTEE MEMBER STERN: Can I do a quick follow-up?

CHAIR LOOMIS: Yeah.

COMMITTEE MEMBER STERN: Yeah, so -- and the other point that I forgot to mention before going back to the issue of the tables is that sometimes, you know, for example here you have a cohort study with a one-time measurement, but it's pre-diagnosis, so that, you know, as epidemiologists that makes us feel good. But as one-time measure, we acknowledge, you may not recapitulate, so therefore we're locked in a situation where we have misclassification which is likely biasing results towards the null.

So if we have multiple studies and they're all showing some association, right, all moving in the right direction, each individual study may not have been super informative, but altogether they're telling a story of what may be going on, which we would miss, as she was saying, if we put it all in a paragraph.

And probably if I had to review that, I would be pulling those papers anyway and looking at them carefully, so they may as well be in the table. So that's why I think we need like a fine line between pushing all the studies into non-informative, which in epidemiology that would be a big majority of studies, or -- unfortunately, or finding out a medium ground that we all feel comfortable as a Committee, right, that we can live with.

CHAIR LOOMIS: Further comments.

Yeah.

COMMITTEE MEMBER BUSH: So not that I'm -- I'm very much in favor of streamlining the HID. Wondering if there's opportunity here to kind of -- this touches on some of the other conversation using, you know, generative AI, or some way of creating a metric or an index of studies. And there's -- I think some of this is done with the key characteristics, but at least binning studies this way with a metric that still gets manually interrogated, right, but using that as a way of creating some -- you know, these are the tier 1 studies or tier 2 studies, something like that, and then, you know, giving us all of that information, but at least then we can use that to maybe delve into the lower ranking studies or what determines, right?

At some point, you're going to have to make a decision of what is least informative. And, you know, unconscious bias is going to enter into that as well. So if there was a metric attributed to that, that -- you know, I'm brainstorming and wondering if there's any initiative or any opportunity there that you're aware of to help categorize these studies.

DR. SANDY: And can you clarify, are you talking about both animal and epidemiologic studies or just one of those types?

COMMITTEE MEMBER BUSH: Both.

DR. SANDY: So with the animal studies, we've tried to give you some sort of a ranking based on study design with some flexibility built in there as well. And I'm pleased to hear Dr. McDonald encourage us to use our judgment to decide, you know, even if it's a knockout mouse, if it's important, to talk about that in detail. I think the trouble is or the difficulty is with epidemiologic studies, there's so many variables that depend on the chemical, and the types of exposure that may occur, and the patterns of exposure, and then the study design, and conduct -- you know, study conduct -- the way it was conducted and reported that it's hard to come up with a metric that will be a one-size-fit-all.

COMMITTEE MEMBER BUSH: May I just quickly?

CHAIR LOOMIS: Go ahead.

COMMITTEE MEMBER BUSH: So I completely agree. And so I guess the question for me is I'm asking is there any motivation or interest from OEHHA or another authoritative body to look into the generative AI capacity to give this kind of a metric. And if there is no answer, I understand. It's probably a no.

DR. SANDY: We don't have that in our sights right now.

COMMITTEE MEMBER BUSH: Right. Big pharma is

using this, right, in their initiatives and their opportunities to look at streamlining. So that's why I brought it up. Thank you.

CHAIR LOOMIS: All right. Other questions, comments from this side of the room?

Dr. Crespi.

COMMITTEE MEMBER CRESPI: I definitely appreciate the effort of trying to streamline the document to make it easier to digest and to evaluate the literature. And I guess I had one comment about in particular summarizing the human studies, and that is that in the tables there seemed to be an emphasis on what the limitations are of the studies, whereas in the past, there was both strengths and limitations. And I feel like perhaps, you know, when a table is developed, it could maybe include, you know, a -- you know, not just give us the negatives, but give us the positives too.

Thank you.

CHAIR LOOMIS: Anymore on this side?

All right. I'll go back over here. Anything else, Dr. McDonald?

COMMITTEE MEMBER McDONALD: Very minor point back on the introduction. I know you give a summary of other authoritative bodies that are listed as part of the law, IARC, NTP, NIOSH, OSHA, and EPA, FDA. I would -- I always

want to hear what the European Commission also is saying. I know that's an add, but I'm always looking for it anyway.

CHAIR LOOMIS: Okay. Anything else on this side? Good there.

Martha, do you feel like you got the kind of input you wanted?

DR. SUN: Yeah, I think so. Points well taken.

COMMITTEE MEMBER STERN: Just a quick comment. I want to say that I always appreciated -- and I remember these from the last document. I can't remember if you did that before too, that you already are separating studies with some intention in terms of informative by study design, because I remember seeing them grouped by, you know, the studies that estimated before or after. So I think there's already some of -- some of that intention that you had with this revision was already there and I appreciated that. I found that helpful.

DR. SUN: Yeah, we did that for PFOS.

COMMITTEE MEMBER STERN: Yes, I remember. Yeah, I found that very helpful, yeah.

CHAIR LOOMIS: Anything else?

Yes.

DR. SANDY: I wonder if there's any possibility of discussing how we might address Dr. Stern's suggestion

of having some early consultation on a particular set of studies and data before the document is developed.

COMMITTEE MEMBER STERN: So one suggestion -- I don't know if these might work with the flow of how you put together these documents, but if you, as a team, came up with a suggestion of sort of how to run studies. These are the most informative, for example, studies that were prospective, that adjusted for X, Y, and Z, that use this type of measurement, and the studies that include one, two, or three of those things, but not all, are, you know, immediately informative and the studies that have none of these are not informative. And maybe you can share that with the Committee and we can kind of provide feedback on that before you start compiling the documents, that could be one way, because as it was mentioned, it's going to very by agent, right?

Each compound is going to have different confounders that we're going to be worried about, so we might have different requirements of what kind of confounders we want to see in the models in the estimates depending on what we're evaluating.

CHAIR LOOMIS: So not to be discouraging, I think that is an interesting idea that moved toward an IARC type of process where the Committee is more involved with developing a written product, but it strikes me as a real

departure from the way this process has worked until now.

I trust the staff to make that determination. And having been on the staff side of it at IARC, I would say, you know, actually it could be -- it could be more stimulating and interesting for the staff to be involved in making those decisions. And I think the Committee can then review what's been done in the way that we have and always have the opportunity to review individual studies if we wish to do so.

That's just my opinion though. I'm not trying to weigh in as Chair here.

Neela.

DR. GUHA: Hi. Thanks. I agree with all of the comments that were made before about the difficulty, about putting the epi studies into different bins, because we all know that there's so many factors that contribute to what an informative study may look like for a particular evaluation. However, for evidence synthesis, I think there's an important concept that a lot of agencies are moving towards is the concept of triangulation, where you can look across a set of studies, most informative and least informative, to come up with a -- with a hazard conclusion. So it's not really throwing away a set of studies, but each set of studies may be informative for forming a causal conclusion.

CHAIR LOOMIS: Thanks.

Other suggestions for dealing with this particular notion?

COMMITTEE MEMBER EASTMOND: I see the challenge, but because all deliberations are done in public as a public meeting, in order to have an interim feedback loop, you would have to schedule additional meetings to give you feedback in a timely fashion or else your hazard identification documents are going to have to span multiple years, which I don't think works well into your scheduling and planning. So I just don't know how this can be done very easily without, you know, putting additional burden on the staff and on the Committee. So that's my thoughts.

CHAIR LOOMIS: Any other suggestions?

No.

All right, Lauren.

DIRECTOR ZEISE: So around -- along the lines of additional feedback. I think we've heard a really good discussion on this. And so we will discuss it further among ourselves and may come up with some idea about how we might get some feedback, but really appreciate all the discussion on this issue.

CHAIR LOOMIS: Okay. Very good.

I think we'll close discussion on this item then

and move on to the last item, which is staff updates. I know that I skipped over the break. If anybody would like to have a break, please let us know and we can consider that, but we're close to the end and -- so we can choose to either finish early or break for a few minutes.

Committee members are saying let's go, so let's go. Staff updates.

(Thereupon a slide presentation).

KIANA VAGHEFI: Hello. Thank you, Dr. Loomis. I'll be providing you with an update on important Proposition 65 developments since the last CIC meeting. I'll start by going over the chemicals or endpoints added to the Proposition 65 list or under consideration for potential listing, as well as chemicals considered but not listed. Then I'll review proposed safe harbor levels. After that, I'll turn it over to our counsel Kristi Morioka to provide a brief update on other regulatory actions.

[SLIDE CHANGE]

KIANA VAGHEFI: Since the Committee's last meeting, seven chemicals have been added to the Proposition 65 list, anthracene, 2-bromopropane, dimethyl hydrogen phosphite, coal-tar pitch, fluoro-edenite fibrous amphibole, and silicon carbide whiskers were add as carcinogens, and bisphenol S was added as a reproductive

toxicant for the female reproductive endpoint.

[SLIDE CHANGE]

KIANA VAGHEFI: BPS remains under consideration for listing as causing developmental and male reproductive toxicity. Information from the BPS data call-in will be used in preparation of a hazard identification document for a future DARTIC meeting on these endpoints.

Additionally, OEHHA issued a data call-in on vinyl acetate to solicit information related to its carcinogenicity. This information is being used in the preparation of a hazard identification document for future consideration by the Carcinogen Identification Committee.

[SLIDE CHANGE]

KIANA VAGHEFI: Since the Committee's last meeting, a no cancer significant risk level was adopted for inhalation exposures to antimony trioxide, which became effective January 1, 2024. We proposed an update to the no significant risk level for exposure to ethylene oxide from 2 micrograms per day to 0.058 micrograms per day for the inhalation route and 1.5 micrograms per day for the oral route. We're still in the regulatory process for this proposal.

And now, I will turn things over to Kristi.

[SLIDE CHANGE]

SENIOR ATTORNEY MORIOKA: I just have a brief

regulatory update. So on October 27th -- or October -- yeah, October 27th, 2023, OEHHA noticed a proposed rulemaking that would amend and add new sections to the Safe Harbor Warning regulations. This proposal would provide information to consumers and disincentivize unnecessary prophylactic warnings by amending existing short form safe harbor warnings that currently say, "Warning, Cancer", and the Proposition 65 website to provide several different options for warnings that include the name of a carcinogen or a reproductive toxicant or both. So, for example, one warning may be, "Warning, Can Expose you to Formaldehyde a Carcinogen," and then the Prop 65 website.

Our regulatory proposal includes a 2-year period for businesses to gradually transition to the new warnings. And the proposal also includes safe harbor status for short-form warning content on food products, clarifications to internet and catalog safe harbor warning requirements, and new tailored warning options for off-highway and motor vehicle parts and recreational marine vessel parts.

We held a public hearing on this proposal and the initial public comment period closed in January of 2024. We are still in the midst of the regulatory process for this proposal.

Do any of the members have any questions?

Yes.

COMMITTEE MEMBER EASTMOND: Could you briefly review the warnings for food, this came up in conversation. I was just curious what the current warnings and what are the proposals?

SENIOR ATTORNEY MORIOKA: Well, sure. The current -- the proposed short-form warnings for food products mirror the proposal for short-form warnings for the short-form proposal in general. And hang on a second, because I need to pull those up really quick.

Let's see, there's two options for carcinogens, two options for reproductive toxicity.

Hold on a second.

DIRECTOR ZEISE: Maybe I could just weigh in a little bit here.

SENIOR ATTORNEY MORIOKA: Oh, sure.

DIRECTOR ZEISE: I think one of the key things is that what we're proposing is that our short-form include the name of the chemical. And so that's really key, so people know what they're being exposed to.

SENIOR ATTORNEY MORIOKA: And then the food warning.

DIRECTOR ZEISE: So we're making that modification. So it would hold for both consumer products

and for food.

COMMITTEE MEMBER EASTMOND: So at the grocery store, there would be a whole list of things, is that the idea?

DIRECTOR ZEISE: So the current -- we'd -- we're following the current regulation, which is you wouldn't identify every single carcinogen. You'd identify at least one carcinogen for which warning would be required. So there are a lot of products where exposures are well below the warning threshold and wouldn't require a warning.

COMMITTEE MEMBER EASTMOND: Okay. Thanks.

SENIOR ATTORNEY MORIOKA: I will -- can I give you all of the food warnings? Can I email all of the food warnings to you afterwards, if that's okay?

COMMITTEE MEMBER EASTMOND: That would be great.

SENIOR ATTORNEY MORIOKA: Thank you.

Any other questions?

Thank you.

CHAIR LOOMIS: Thank you, Kristi. Thank you, Kiana. That brings us to that last agenda item. And Dr. Zeise has kindly offered to try to summarize the meeting content since there were no Committee actions.

DIRECTOR ZEISE: Sorry. Yeah. So normally, I would summarize the Committee actions. And this wasn't in -- this wasn't a meeting that had Committee

actions. We had a lot of great input on the discussion on human variability and sensitivity due to polymorphisms. And really want to thank the speakers for their presentations, really thought provoking.

I think we saw that -- examples of cases where -- some pretty influential polymorphisms. And in those cases, some discussion around how we might take those more into account. And I think we'll be thinking about how we might take those into account as we write up hazard identification documents as well. So really good discussion there and we appreciate that.

Then turning to the streamlining of hazard identification documents, I think we heard general support for streamlining these documents, but with a lot of caveats. For the introductory sections, I think we heard general support, but there was one item that I wrote down for further inclusion. It's in our notes and it's escaping me right now.

COMMITTEE MEMBER McDONALD: Europe.

DIRECTOR ZEISE: Adding Europe. That's right, adding the European Union findings to the discussion where we talk about what other authoritative bodies have done. And then for the human, a much more complex set of data that makes streamlining -- we have support for streamlining, but with a caution, because of the very

varied data sets. And the type of information that might be available on a specific chemical might be very, very different from other chemicals. So a lot of cautionary notes about binning with respect to, for example, study design. So I think we heard that loud and clear.

And then for the animal data again, I think not as complex data sets. General support for the way we've been tabulating data. But I think we did hear support for not going into a lot of detail on some of the studies that aren't really influential for the Committee's decision.

And then -- yeah, so I think that about does it. And you heard the updates. And we can send to the full Committee -- I think we are sending to the full Committee our regulations as we develop them, but we'll make sure that you do get our short-form regulations, so you have that. So, okay with that, I just want to thank everyone on the Committee for coming to this meeting, having the discussion, providing us your input, taking the time to do that. We hope to have a Committee meeting later in the year to tackle with -- tackle a particular chemical listing. And I think the discussion today has really informed as we pull those materials together, so really appreciate that.

And I want to thank staff for all the work in putting this meeting together and including our

implementation staff and IT staff, scientific staff, and legal staff. So it takes a village to do these meetings. And then very special thanks to our speakers as well. Really appreciate it. And with that, I'll turn it back over to Dana -- Dr. Loomis.

CHAIR LOOMIS: Thank you. Well, I'll just second all the thanks to the Committee for giving valuable time to attend the meeting today, staff for preparing all the materials that we've had in front of us for discussion and consideration, and to the speakers for their very valuable input to this process. And with that, I'm happy to adjourn the meeting and allow those Committee members with afternoon flights to get out of here.

(Laughter).

CHAIR LOOMIS: So thanks everybody. The meeting is adjourned.

(Thereupon the Carcinogen Identification Committee adjourned at 2:19 p.m.)

CERTIFICATE OF REPORTER

I, JAMES F. PETERS, a Certified Shorthand Reporter of the State of California, do hereby certify:

That I am a disinterested person herein; that the foregoing California Office of Environmental Health Hazard Assessment, Carcinogen Identification Committee was reported in shorthand by me, James F. Peters, a Certified Shorthand Reporter of the State of California, and thereafter transcribed under my direction, by computer-assisted transcription;

I further certify that I am not of counsel or attorney for any of the parties to said workshop nor in any way interested in the outcome of said workshop.

IN WITNESS WHEREOF, I have hereunto set my hand this 12th day of March, 2024.

JAMES F. PETERS, CSR
Certified Shorthand Reporter
License No. 10063