MEETING

STATE OF CALIFORNIA

ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

PROPOSITION 65

CARCINOGEN IDENTIFICATION COMMITTEE

JOE SERNA JR.

CALEPA HEADQUARTERS BUILDING

1001 I STREET

SIERRA HEARING ROOM

SACRAMENTO, CALIFORNIA

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A P P E A R A N C E S

COMMITTEE MEMBERS:

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Jason Bush, Ph.D.

Shanaz Dairkee, Ph.D.

David A. Eastmond, Ph.D.

Joseph Landolph, Ph.D.

Peggy Reynolds, Ph.D.

Luoping Zhang, Ph.D.

STAFF:

Dr. Lauren Zeise, Director

Mr. Allan Hirsch, Chief Deputy Director

Ms. Carol Monahan Cummings, Chief Counsel

Dr. Jennifer Hsieh, Reproductive and Cancer Hazard Assessment Branch, Cancer Toxicology and Epidemiology Section

Dr. Gwendolyn Osborne, Reproductive and Cancer Hazard Assessment Branch, Cancer Toxicology and Epidemiology Section

Dr. Karin Ricker, Reproductive and Cancer Hazard Assessment Branch, Cancer Toxicology and Epidemiology Section

Ms. Michelle Ramirez, Proposition 65 Implementation

Dr. Martha Sandy, Chief, Reproductive and Cancer Hazard Assessment Branch

Dr. Meng Sun, Reproductive and Cancer Hazard Assessment Branch, Cancer Toxicology and Epidemiology Section

A P P E A R A N C E S C O N T I N U E D

ALSO PRESENT:

Dr. Jay Murray, International Fragrance Association North America

I N D E X

P R O C E E D I N G S

DIRECTOR ZEISE: Welcome, everyone. I'd like to welcome you to this meeting of the Carcinogen Identification Committee. I'm Lauren Zeise the Director of the Office of Environmental Health Hazard Assessment. And before I turn this meeting over to Chairman Mack, I'd like to cover just a few logistics, as well as introduce the Panel and the staff

So first, the meeting is being transcribed and webcast. So I just want to remind everyone to speak clearly into the mics and give your name for the record.

With respect to logistics, drinking fountains and restrooms are located out the back door, and you turn left, go to the end of the hall. In the event of a fire alarm or any another reason to evacuate, just take the stairs out down, and go out the doors of the building and we'll relocate at a site across the street. And we'll be staking breaks periodically for our court reporter.

Now, I'd like to introduce the Carcinogen Identification Committee. Dr. Mack to my left, then at the far end Dr. Jason Bush, Associate Professor, Cal State University, Fresno; Luoping Zhang, Associate Adjunct Professor, School of Public Health at the University of California, Berkeley; then David Eastmond, Professor and Chair, Department of Cell Biology and Neuroscience,

University of California, Riverside; to my right Dr. Joseph Landolph, Associate Professor, University of Southern California; to his right Dr. Peggy Reynods, Senior Research Scientist at the California Prevention Institute of California, and consulting professor at Stanford University School of Medicine; and then Dr. Shanaz Dairkee, senior scientist, California Pacific Medical Center. So welcome, everyone.

And then the OEHHA staff, Allan Hirsch Chief Deputy Director; Carol Monahan Cummings, Chief Counsel; Dr. Martha Sandy, Branch Chief of the Reproductive and Cancer Hazard Assessment Branch; Karin Ricker, staff toxicologist, RCHAB; Gwen -- Gwendolyn Osborne, M.P.H., staff toxicologist, RCHAB; Meng Sun, staff toxicologist, RCHAB; and Jennifer Hsieh, staff toxicologist, RCHAB; Julian Leichty, part of the Prop 65 Implementation group; Esther Barajas-Ochoa with the Implementation staff, and Michelle Ramirez with the Implementation staff, and Rose Schmitz with RCHAB.

And so welcome, everyone. And now I'm going to ask Carol to give some introductory remarks.

Carol Monahan Cummings our Chief Counsel.

CHIEF COUNSEL MONAHAN CUMMINGS: Good morning. So at each meeting, I just do a quick reminder on a couple of issues. First, I wanted to remind you that in your

materials in the last tab is the criteria for listing that was adopted by this Committee several years ago. If you have questions about whether or not a particular decision to list should be made, then you should look at that criteria. The criteria does not include consideration of future impacts of a listing, for example, whether warnings would be required or particular products might be affected. You may hear about that, but it's not really part of the listing criteria.

What you're asked to do is find whether or not a chemical has been clearly shown through scientifically valid testing, according to generally accepted principles, to cause cancer. That's a standard of scientific -- it's a scientific judgment call not a legal standard of proof.

This Committee can decide to list a chemical based entirely on animal evidence. The chemical need not have been shown to be a human carcinogen. You don't need to consider whether current human exposures to the chemical are sufficiently high enough to cause cancer. This -- the members of this Committee are very well qualified, were appointed to the Committee by the Governor because of your scientific expertise, and are considered the State's qualified experts on carcinogenicity of given chemicals. So you don't need to feel compelled to go outside that charge.

In the event you feel you have insufficient information or need more time to think about the question or discuss it, there's no requirement that you make a decision today. Feel free to ask clarifying questions of me or the other OEHHA staff during the meeting. If we don't know the answer to your question, we'll do our best to find out and report back to you.

Do you have any questions at this time?

COMMITTEE MEMBER EASTMOND: I have a question.

In public comments, apparently there's one of the interpretations of this law has to do with chemicals naturally found in foods. Could you describe that kind distinction?

CHIEF COUNSEL MONAHAN CUMMINGS: Sure. The reference is to a regulation that our office adopted many years ago that has to do with chemicals that occur naturally in foods. And it's a exemption from the warning requirement that is a little bit complicated. It's a fairly long regulation, but it only applies once a chemical is listed, and it only applies to those chemicals that are naturally occurring in a particular food.

So it's true there is an exemption, but it's not something that would be an issue for you all today.

DIRECTOR ZEISE: All right. So now I'll turn the meeting over to the Chair Dr. Thomas Mack, Professor,

School of Medicine University of Southern California.

Dr. Mack.

CHAIRPERSON MACK: Welcome from me. And let's get started. First thing I guess I should say is anybody who wants to make comments during the -- from the public, and I see all my friends out there, feel free to do so, but go find yourself a blue card, and sign up, and get ready, and then we'll do it when the time comes.

But I'm sure that nobody is going to have any problems with anything that's said today as usual.

(Laughter.)

CHAIRPERSON MACK: All right. Thank you very much. And now we start with the staff.

(Thereupon an overhead presentation was presented as follows.)

DR. SANDY: Thank you, Dr. Mack. This is Martha Sandy. I will just introduce my staff that will be making this presentation, and just clarity that as you see in the hazard identification document, there's a number of staff that were authors of this, but we'll have four staff making the presentation. We've tried to give a summary overview of the document. We can't possibly go through everything in the document.

And first, we'll be hearing from Dr. Hsieh and then Dr. Osborne, then Dr. Ricker, and then Dr. Sun.

So I'll turn it over to Dr. Hsieh.

DR. HSIEH: Thank you, Dr. Sandy.

Good morning I'm Jennifer Hsieh. And today, we are here to present a summary overview of the evidence on carcinogenicity of coumarin.

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DR. HSIEH: Coumarin is a lactone and most specifically it is a benzopyrone. The chemical structure of coumarin is shown here in this figure with carbon number labeled and lactone structure circled in green. Coumarin is a single compound with a specific CAS number. Coumarin is not the same and should not be confused with other compounds like are sometimes referred to as "coumarins", that have a different chemical structure.

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DR. HSIEH: Source of coumarin. Coumarin occurs in many plants such a tonka beans, cinnamon, and sage. Some essential oils also contain coumarin. Coumarin also can be extracted from plant or synthesized commercially. Coumarin has a pleasant sweet odor. It may be used as a fragrance enhancer in perfume and cosmetics, as flavoring additive in tobacco product, and to mask odor in some plastics and paints.

Coumarin is not approved for use as a drug in United States, although in 1990s, it was the subject of a

clinical trial as a potential cancer treatment. FDA banned the use of coumarin as a direct food additive in 1954, because of severe hepatotoxicity in animals.

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DR. HSIEH: Coumarin has been reviewed by International Agency for Research on Cancer, or IARC, and European Food Safety Authority, or EFSA. IARC classified coumarin, “Not classifiable as to its carcinogenicity to humans”, based on no epidemiological data and limited evidence in animals.

EFSA also reviewed coumarin and identified it as a carcinogen in rats, and possibly in mice in 1994. EFSA based its total Tolerable Daily Intake on hepatotoxicity.

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DR. HSIEH: And this slide provides an overview of this presentation on the evidence on coumarin carcinogenicity.

There were no human cancer studies. Therefore, the presentation will begin with discussion of carcinogenicity studies in animals. That will be followed by a presentation on human relevance, including on pharmacokinetic metabolism CYP2A6 polymorphism, hepatotoxicity, and common biological pathway identified from toxicogenomic data. Then, mechanistic study organized by IARC's key characteristics carcinogen will be

presented.

In the case of coumarin, this study covered data on genotoxicity, electrophilicity of its metabolites and oxidative stress. Numerous studies on CYP2A6 genetic polymorphism and several studies on toxicogenomics, that are new since IARC 2000 review, will be discussed.

Finally, we will conclude with a summary of evidence.

Now, Dr. Osborne will present the data on animal carcinogenicity.

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DR. OSBORNE: So animal carcinogenicity studies include 7 rat studies, 4 mouse studies, and 2 hamster studies.

Evidence of tumorigenicity comes primarily from these 8 studies in rats and mice, which I'll be talking about more in the following slides.

So in the 3 rat studies that I've highlighted here, our studies have limited study -- limited study design or reporting. The first one by Evans et al., 1989 had one dose level with only 5 animals examined at the conclusion of the study at 78 weeks, and was not well reported.

The study by Baer & Griepentrog and Griepentrog 1973 were both published in German, and reported bile duct

carcinomas in several rats. In later reviews by other authors, these tumors were described as non-neoplastic cholangiofibrosis.

The third study, by Hagan et al., reported in 1967 had between 5 and 7 animals of each sex per dose group. It reported liver damage as focal proliferation of bile ducts with cholangiofibrosis, fatty change, and focal necrosis. This study did not separately report findings from males and females and was inadequately reported. These studies will not be discussed further in this presentation.

At this time, I'd also like to mention the hamster studies here. Coumarin was administered in feed at levels of 0.1 percent and 0.5 percent to -- for 2 years to males and females. Two uncommon pancreatic islet cell carcinomas were seen in females in the high dose group. Overall, the survival of this study is limited by the small numbers of animals per group and poor survival.

In the following slides, I'm going to present the details of the rat and mouse studies by NTP and Carlton et al., that I have highlighted here.

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DR. OSBORNE: In an NTP study, male F344/N rats were administered coumarin by gavage for 5 days per week for 103 weeks at doses of 25, 50, and 100 milligrams per

kilogram.

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DR. OSBORNE: Rare renal tubule adenomas and combined adenomas and carcinomas were observed in the mid-dose group by pairwise comparison. Also, two uncommon renal tubule oncocytomas were observed in the low-dose group.

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DR. OSBORNE: NTP 1993 also conducted a stop exposure evaluation in male rats. In this study, groups of male rats were given 100 milligrams per kilogram coumarin via gavage for 9 or 15 months. Some of the rats were sacrificed at the end of exposure while others were kept on study, receiving only the corn oil vehicle via gavage until the end of the 103 weeks. The continuous exposure 100 milligram per kilogram dose group is shown here for comparison.

At the end of the 103-week study, a statistically significant increase in the incidence renal tubule adenomas was observed in the 9-month stop exposure group by pairwise comparison with controls. In the 15-month stop-exposure group, 2 renal tubule adenomas were observed at the end of the 103-week study. Among the animals in the 15-month exposure group that were sacrificed right at 15 months when exposure stopped, one additional renal

tubule adenoma was observed.

Finally I'll note that 2 rats in the 15-month stop-exposure group also had uncommon renal tubule oncocytomas which were observed at the end of the 103-week study.

NTP considered all these findings and concluded that male rats have some evidence of carcinogenic activity.

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DR. OSBORNE: In female F344/N rats administered coumarin via gavage for 5 days per week for 103 weeks in an NTP study, a few rare renal tubule adenomas were observed in the mid- and high-dose groups with a significant trend. NTP considered this to be equivocal evidence of carcinogenic activity in female rats based on a marginally increased incidence of renal tubule adenomas.

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DR. OSBORNE: Carlton et al., 1996 administered coumarin in feed to male Sprague-Dawley rats for 2 years. The 3 lowest dose groups were administered coumarin while in utero and throughout the lifetime, while the 2 higher dose groups were administered coumarin starting after weaning.

The study reported tumors as non-metastasizing and metastasizing cholangiocarcinomas, both of which were

increased in the highest dose group compared to controls, as well as hepatocellular adenomas or carcinomas combined.

The authors proposed that the liver tumors were due to exceedance of the maximum tolerated dose that led to hepatotoxicity. Body weight gain was decreased in the 3 highest dose groups in the study, but this by itself is not indication of an excessive high dose. In deed, survival in the 2 highest dose groups was actually better compared to controls.

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DR. OSBORNE: Carlton at al., also conducted a study on female Sprague-Dawley rats with a similar study design as that in male rats, where the first 3 dose groups were administered coumarin starting in utero and the two highest dose groups received coumarin starting only after weaning. Similar to the study in males multiple types of live tumors were observed in female Sprague-Dawley rats.

Non-metastasizing cholangiocarcinomas and hepatocellular adenomas or carcinomas were significantly increased in the highest dose group compared to controls. Similar to male rats, the observations of increased survival in the 2 highest dose groups compared to controls and decreased body weight gain do not support the conclusion that the liver tumors were the result of excessive toxicity.

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DR. OSBORNE: In 2-year gavage studies conducted in male mice by the NTP, lung and forestomach tumors were observed. There was significant increases in alveolar/bronchiolar adenomas, and adenomas and carcinomas combined in the high dose group with a significant trend. One and 2 rare forestomach cell carcinomas were observed in the low-dose and mid-dose groups.

Forestomach papillomas and carcinomas combined were significantly increased in the low-dose group by pairwise comparison with controls.

NTP considered this to be some evidence of carcinogenic activity in male mice based on increased incidence of alveolar/bronchiolar adenomas.

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DR. OSBORNE: In the NTP female mouse study, lung, liver, and forestomach tumors were observed. There was significant increases in alveolar/bronchiolar adenomas, carcinomas, and adenomas and carcinomas combined in the high-dose group and by trend. Significant increases in hepatocellular adenomas and adenomas and carcinomas combined were seen in the low- and mid-dose group.

There was one forest -- rare forestomach carcinoma in the low-dose group and one in the mid-dose

group. NTP considered this to be clear evidence of carcinogenic activity in female mice based on increased incidences of alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, and hepatocellular adenomas.

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DR. OSBORNE: In a 2-year feeding study in male CD-1 mice by Carlton et al., lung tumors were observed. There were significant increases in alveolar/bronchiolar carcinomas in the high dose group with a significant trend. The 2000 IARC summary of this study noted an unpublished company report analyzing mortality-adjusted tumorage -- tumor rates, which found no treatment-related increases in these lung tumors.

We have relied on the information in the published study by Carlton et al., which includes a statement that survival treated male mice was similar to that of controls.

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DR. OSBORNE: In the female CD-1 mouse study by Carlton et al., liver tumors were observed. There was a significant increase in the incidence of hepatocellular adenomas or carcinomas in the low-dose group.

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DR. RICKER: OEHHA identified 4

co-carcinogenicity studies. They're all short-term rodent studies ranging from 16 to 28 weeks duration. Three studies were conducted with DMBA, one with benzo(a)pyrene.

Coumarin was administered prior to and concurrent with either DMBA or BP. One specific tumor type was evaluated in each study as noted on this slide.

In all studies, co-administration with Coumarin reduced tumor formation compared to either DMBA or BP alone. It is possible that there may be metabolic competition between coumarin and BP or DMBA. Coumarin and BP are both metabolites by the same CYP enzyme CYP2A5.

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DR. RICKER: We will now discuss the pharmacokinetics and metabolism of coumarin. We start with an overview of the human and animal studies that we identified followed by a brief description of absorption, distribution, and elimination. We will then describe in more detail the metabolic pathways and metabolites of coumarin.

As you can see on this slide, several in vivo human metabolism studies were identified, and include multiple routes of exposure. We also identified human in vitro studies that were conducted with liver microsomes, liver slices, and recombinant enzyme preparations.

In vivo animal studies were conducted with a wide

range of species and via multiple routes. They were also numerous in vitro studies, including studies with skin, liver slices, liver microsomal and cytosolic fractions, and recombinant enzyme preparations.

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DR. RICKER: Coumarin is extensively and rapidly metabolized. The data presented here are from human studies. Absorption of coumarin is generally fast. About 60 percent of coumarin applied to skin is absorbed within 6 hours. Distribution occurs throughout the body, and the plasma half-life of coumarin has been reported to be between 1 to 1.7 hours following oral, dermal, or IV routes.

Coumarin is largely excreted in metabolized form, and hence very little coumarin is excreted unchanged. Primary excretion occurs via urine, and about 95 percent of coumarin is excreted in 4 hours of after oral administration. Excretion is somewhat slower after dermal applications. There's very little biliary excretion in humans. Fecal excretion has been measured only following dermal exposure and amounted to 1 percent of the applied dose in 120 hours.

By contrast, biliary excretion is higher in some animals. Up to 38 percent has been reported in rats, and about 12 percent in hamster.

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DR. RICKER: Coumarin metabolism is similar in humans and animals. There are 2 main pathways, 7-hydroxylation and 3,4-epoxidation. When coumarin is hydroxylated at the 7 position, it yields 7-highdroxycoumarin. This reaction is catalyzed primarily by the enzyme CYP2A6 shown here in the red box. The 7-hydroxycoumarin is excreted directly or can be conjugated with glucuronic acid or sulfates prior to excretion.

The second main pathway is to epoxide pathway, in which coumarin is metabolized to coumarin 3,4-epoxide or CE for short. The epoxide spontaneously forms ortho-hydroxyphenylacetaldehyde, ortho-HPA for short, after ongoing ring-opening of the lactone ring and decarboxylation. These 2 metabolites, coumarin epoxide and ortho-HPA are reactive electrophilic metabolites.

Ortho-HPA can be further oxidized by aldehyde dehydrogenase to o-hydroxyphenylacetic acid, ortho-HPAA, or it can be reduced to ortho-hydroxyphenylethanol, ortho-HPE. Ortho-HPE in turn can be oxidized back to ortho-HPA, thus replenishing the pool of ortho-HPA.

Instead of undergoing further oxidation and reduction reactions, coumarin 3,4-epoxide can also be detoxified with glutathione and be further metabolized to

coumarin 3-mercapturic acid. As some products have been observed in animals, but have not yet been looked for in humans, they're shown here in bright blue.

In other minor pathways, coumarin can be hydroxylated at other carbon positions, yielding a variety of hydroxy coumarins shown here. It can also be metabolized to ortho-coumaric acid, which in turn can form 4-hydroxycoumarin and ortho-hydroxyphenylpropionic acid.

It is unclear if human gastric intestinal microbes can biotransform coumarin in the gut to form 3,4-dihydrocoumarin and ortho-hydroxyphenylpropionic acid as has been shown in rats.

I would like to come back now to the epoxidation pathway shown here in the large red box, and talk a little bit about toxicokinetics and the formation and clearance of the electrophilic metabolites CE and ortho-HPA.

There's some indication from in vitro studies that differences in the kinetics of ortho-HPA formation and subsequent oxidation to the acetic acid, as well as detoxification reactions may determine the ultimate toxic effects of these metabolites.

Mice appear to catalyze the oxidation of ortho-HPA to ortho-HPAA in the liver more efficiently than rats, which is evidenced by the amount of ortho-HPAA formed in mice, which can be up to 41 percent of the

administered dose but is only 12 percent in rats.

Mice also have a faster clearance rate for the oxidation of ortho-HPA to ortho-HPAA compared to rats. Lastly, while both mice and rats reduce ortho-HPA to ortho-HPE, this is only a major reaction in rats.

It has been suggested that a cycle of oxidation reduction from ortho-HPA to ortho-HPE and back may contribute to slower hepatic clearances of the toxic aldehyde in the rat.

Furthermore, the extent and kinetics of additional detoxification reactions, such as conjugation with glutathione may also determine the extent to which electrophilic metabolites bind covalently with cellular macromolecules in a given tissue.

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DR. RICKER: The purpose of this slide is to point out the importance of the genetic polymorphisms of the human CYP2A6 enzyme here in the red box to the overall coumarin metabolism in humans. In some, but not all, humans, the 7-hydroxylation pathway is the main pathway of coumarin metabolism.

The human CYP2A6 is a highly polymorphic enzyme and hence the metabolic pathway is primarily determined by an individual CYP2A6 genetic variant. This is evidenced by the wide differences in amounts of 7-hydroxycoumarin

versus ortho-HPA measured as the acetic acid, excreted in human urine by different people.

In some individuals, 7-hydroxycoumarin can constitute up to 92 percent of urinary metabolites. Conversely, in an individual who is homozygous for a loss-of-function CYP2A6 variant allele, the amount of 7-hydroxycoumarin measured in the urine can be less than 0.02 percent of the applied dose, while the amount of ortho-HPAA accounts for nearly 55 percent of the total urinary metabolites.

Clearly, this is a metabolic shift that allows for greater formation of electrophilic metabolites. We will now hear more about the CYP2A6 polymorphism, its distribution in human population, and its implications for human health risk assessment in the next few slides.

Dr. Sun will take over.

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DR. SUN: As we mentioned in the metabolism slides, in humans CYP2A6 is the main enzyme for coumarin 7-hydroxylation. 7-hydroxylation is considered a detoxification reaction compared to the epoxidation pathway in which electrophilic reactive metabolites are formed. CYP2A6 is a highly polymorphic gene. To this date, there are at least 45 allele variants with many subtypes within each designated allele.

The distribution of these alleles varies greatly across different ethnicities and populations around the world making certain individuals more susceptible to loss of the enzyme function of CYP2A6. The different allelic sequences result in different levels of enzyme activity. Individuals with decrease-of-function or loss-of-function alleles can be poor coumarin 7-hydroxylators.

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DR. SUN: This table summarizes the CYP2A6 variants reported in the literature. The first column lists the alleles, the second column lists their coumarin 7-hydroxylation activity compared to the wild-type enzyme, and the third column shows the types of genetic changes that lead to the polymorphisms.

Allele A or 1A is considered the wild-type and codes for the fully functional enzyme. Compared to the wild-type, there are alleles that have increased activity, similar activity, decreased activity, or no activity.

For several alleles, their coumarin 7 hydroxylation activity is still unknown, because it hasn't been tested. The genetic changes listed in the third column include gene conversions, duplications, and single nucleotide polymorphism, or snip.

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DR. SUN: This slide shows you an example of the

genotype-phenotype correlation in three studies conducted in the Thai population. The investigators determined the CYP2A6 genotype of human volunteers, gave them each a coumarin tablet orally and measured their urinary excretion of 7-hydroxycoumarin or its conjugate.

The first study -- in the first study 4 out of 192 volunteers were homozygous for Allele 4. In the second study, 4 out of 120 had this genotype, and in the third study, 1 out of 194 had this genotype.

Individuals homozygous for Allele 4 in these 3 studies excreted an average of between 1 percent and 15 percent 7-hydroxycoumarin compared to the wild-type. This gives you an idea of the consequence of carrying 2 copies of a loss-of-function allele.

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DR. SUN: This slide illustrates the distribution of 2 CYP2A6 alleles reported in different populations around the world. Allele 4, which is a deletion allele, and leads to no enzyme activity is shown in green. Allele 9, a decrease-of-function allele is shown in orange. The X axis lists the populations that were genotyped, and the Y axis is the percentage found in each population in the genotyping studies.

Each bar represents a range of frequencies found in the population based on multiple studies with the

bottom of the bar starting at the minimum of the range and the top of the bar showing the maximum of the range. A dot means the frequency came from one study.

Overall, there is a diverse distribution of these 2 alleles. Going from the left, you can see that the frequencies in African individuals and African North Americans are similar as shown by the overlapping of the first 2 green bars for Allele 4, and the first 2 orange bars for Allele 9. Between East or Southeast Asians and Asian North Americans, the frequencies for Allele 4 also over lap and go up to over 22 percent. The lack of the orange bar for Asian North Americans means Allele 9 was not tested in this population.

The rest of the population shown here contain different levels of these 2 alleles. Defective CYP2A6 alleles are present in all of these populations tested, and the carriers of these alleles are the subpopulations that may lose part of their coumarin 7 hydroxylation activity or even all of it.

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DR. SUN: This slide presents data from a newly published study by Zhou et al on the distribution of 176 different cytochrome P450 alleles in over 56,000 unrelated individuals. CYP2A6 was 1 of 12 genes analyzed. Sequencing data came from Exome Aggregation Consortium,

and linkage information came from the 1000 genomes project. Exome sequencing doesn't provide information on the deletion alleles, such as Allele 4 and 5, and duplication alleles.

So in those cases, the authors used frequency data from published literature. Using a different color for each allele, this figure shows the relative contribution of different variant alleles in the five major populations tested. The pie charts do not include the wild-type allele.

The different color combination for each pie chart represents the genetic variation from one population to another. We can see the most frequent variant for Europeans is Allele 35, for Africans it's allele 17, and for East Asians, South Asians, and admixed Americans of Mexican and South American industry, it's Allele 9.

These 3 are all decrease-of-function alleles for coumarin 7 hydroxylation. CYP2A6 variants 2 and 4 are loss-of-function alleles and others shown here are decrease-of-function alleles for coumarin 7 hydroxylation, except for Alleles 14, 21, and 28. CYP2A6 polymorphism is an active research field with many new studies being published each year. Further information on frequencies of CYP2A6 variants is provided in appendix B of the hazard identification document.

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DR. SUN: To conclude, certain CYP2A6 polymorphisms lead to the metabolic shift towards epoxidation, and production of the reactive electrophilic metabolites. Coumarin 3,4-epoxide and ortho-HPA, which combined to cellular macromolecules. Evidence for the shift is seen in human in vivo and in vitro studies as we further discuss in the hazard identification document.

Besides polymorphisms, CYP2A6 activity can also be compromised by non-genetic factors, such as diet or drugs, and can be saturated by exposure to high-dose coumarin. Carriers of loss- or decrease-of-function alleles may be more vulnerable to coumarin toxicity, mediated by the reactive metabolites of the epoxidation pathway.

A number of clinical trials and case reports with coumarin observed hepatotoxicity in the hepatotoxicity in a significant fraction of the people treated. The extent to which this involved loss- or-decrease-of-function CYP2A6 alleles was not well studied.

Next Dr. Hsieh will take over.

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DR. HSIEH: Thank you.

Now, let's switch gears to -- I'm sorry -- mechanistic data. I'll start with genotoxicity data

followed by toxicogenomic data. Coumarin has tested positive for a number of genotoxicity endpoint in studies in bacteria, fungi, cell-free systems, plant cells, mammalian cell in vitro.

While coumarin is generally negative in salmonella, it induce base-pair substitution mutations in the presence of metabolic activation in salmonella strain TA100 in multiple study, and was also positive in another modified strain of TA7002, which detect T:A to A:T transversions.

Coumarin did not induce HPRT or GPT locus mutations in Chinese hamster ovary cell, but it induced chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cell and in onion root tip cells.

Coumarin induced micronuclei formation in human lymphocytes and in two studies using human hepatoma cell line.

Coumarin did not induce unscheduled DNA synthesis in human liver slices in one study, but in aspergillus, coumarin-induced Chromosome instability.

In E. Coli, coumarin did not cause DNA damage, but it inhibited DNA excision repair.

In cell free system, coumarin has been shown to bind to single- and double-stranded calf thymus DNA

However, in in vivo study, no positive

genotoxicity finding has been reported for the four genotoxicity endpoints assessed to date: Sex linkage recessive lethal mutations in drosophila, micronuclei formation in mice, unscheduled DNA synthesis in rat liver cells, and in one unpublished report, DNA covalent binding in rat liver and kidney.

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DR. HSIEH: Four coumarin metabolites has been tested in limited number of genotoxicity assays. The two most electrophilic metabolites coumarin 3,4-epoxide and ortho-HPA have not been tested. Positive finding has been reported for 2 coumarin metabolites: 7-hydroxycoumarin and 3,4-dihydrocoumarin.

7-hydroxycoumarin did not induce mutations in salmonella or unscheduled DNA synthesis in rat hepatocytes, but it did:

Induce expression of the ada DNA repair gene in E. Coli; was weakly positive in the induction of a chromosome aberration in Chinese hamster ovary cell; formed DNA cycloadducts with thymine and cytosine and DNA interstrand crosslinks in synthesized DNA after photoirradiation.

3,4-dihydrocoumarin did not induce mutation in salmonella, and did not induce chromosome aberration in Chinese hamster ovary cell or micronuclei formation in

mice, but it did induce sister chromatid exchange in Chinese hamster ovary cell. 6,7-dihydroxycoumarin and ortho-HPAA were tested in only 3 types of assays and each were negative.

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DR. HSIEH: Moving on to toxicogenomic study. Toxicogenomic data are new since 2000 IARC review. The data sources are from 6 studies conducted in rodents in 2 in vitro study using human hepatocytes. Several of these studies reported that coumarin alters 2 cancer-related biological processes or pathway, namely pathways related to glutathione metabolism and oxidative stress response. OEHHA conducted a gene ontology or GO and Kyoto encyclopedia of genes and genomes, or KEGG, pathway analysis using the microarray data from one rat liver in vivo study. This analysis identified multiple cancer-related biological processes or pathway altered by coumarin.

When we compared these pathways identified in our analysis of the in vivo rat liver study with altered cancer-related pathways reported in one of in vitro human hepatocytes, we identified several common cancer-related pathway altered by coumarin in both rat liver in vivo and human hepatocytes in vitro. These common cancer-related pathways include those related to nucleic acid binding,

metab -- metabolism of xenobiotics by CYP enzyme, and oxidoreductase activity.

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DR. HSIEH: The slide lists the essential cancer-related pathways enriched by coumarin generated from OEHHA's GO and KEGG pathway analysis using microarray data in rat liver in vivo. The left column list the pathway that were enriched by coumarin treatment, and the right column shows their link to one or more of the key characteristics of carcinogen identified by IARC. The pathway highlight in yellow are enriched in both rodents and human.

The pathways listed in the top part of the table are linked to 3 critical carcinogenic characteristic, electrophilic metabolites. The corresponding pathways are metabolism of xenobiotics by CYP enzymes, nucleotide binding. Genotoxic: the corresponding pathways are nucleotide binding, base excision repair and DNA replication. And inducing oxidative stress: the corresponding pathways are glutathione metabolic process, oxidation-reduction process and response to oxidative stress.

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DR. SUN: This slide summarizes other mechanistic studies. There are data on reactive oxygen species

production and glutathione depletion. In addition to the toxicogenomic data that we just heard, traditional toxicology studies have shown that coumarin increases reactive oxygen species production.

In addition, 6,7-DIHYDROXYCOUMARIN, a coumarin metabolite, was shown to increase mitochondrial reactive oxygen species in HeLa cells. The depletion of glutathione has been observed in rat liver in vivo, in freshly isolated rat hepatocytes, and in primary rat hepatocyte cultures.

In addition, the formation of coumarin metabolite-derived glutathione conjugates has been demonstrated in human liver microsomes. The effects of coumarin on cell proliferation is not clear. In one study, coumarin increased the mitotic index of rat hepatocytes by 1.4-fold. However, many other studies have shown that coumarin and its metabolite 7-hydroxycoumarin inhibited cell proliferation and induced apoptosis.

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DR. SUN: In the next two slides we will give you a summary of evidence starting with animal studies. There were multiple tumor findings in rats and mice. The first tumor type is renal tubule tumors seen in male and female F344/N rats. These renal tumors, while mostly benign in the coumarin studies, are rare in rats.

There were also hepatocellular tumors seen in male and female S-D rats, and in two strains of female mice, B6C3F1 and CD-1 mice in the low-dose group.

Liver cholangiocarcinomas were also observed in male and female S-D rats. In the male rats, significant increases were seen in both metastasizing cholangiocarcinomas, and non-metastasizing cholangiocarcinomas.

Lung tumors, specifically alveolar/bronchiolar tumors were seen in male and female B6C3F1 mice, and in male CD-1 mice.

Lastly, increases in forestomach tumors, namely combined squamous cell papillomas and carcinomas, were observed in the low-dose group of male B6C3F1 mice. Forestomach squamous cell carcinomas are rare in male mice.

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DR. SUN: This slide summarizes evidence related to coumarin's possible mechanisms of action and its human relevance. There is evidence to support three possible mechanisms of action.

First, coumarin forms the electrophilic metabolites, coumarin 3,4-epoxide and ortho-HPA, which have been shown to bind covalently to microsomal proteins in rats and humans. These metabolites and their

subsequent clearance and detoxification reactions may play a role in coumarin toxicity, based on data from in vitro studies.

Coumarin can also induce oxidative stress. It depletes cellular glutathione as a result of the formation of coumarin metabolite-derived glutathione conjugates. This reduction or depletion of the glutathione pool may shift the cell's redox balance and impact the cell's overall ability to detoxify additional reactive oxygen species leading to oxidative stress. Evidence for increases in reactive oxygen species comes from studies in HeLa cells as well as in vivo and in vitro toxicogenomic studies.

The third possible mechanism is genotoxicity. As we've heard from Dr. Hsieh, coumarin has tested positive in a number of in vitro and cell-free genotoxicity assays.

Finally, we'd like to present a summary of the evidence regarding the human relevance of coumarin's carcinogenicity.

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DR. SUN: The primary enzyme for coumarin 7 hydroxylation in humans is the highly polymorphic enzyme CYP2A6. Populations around the world carry certain allelic variants of this enzyme that are associated with either no enzyme function or reduced function.

When coumarin 7 hydroxylation by CYP2A6 is compromised, the metabolic shift leads to increased generation of coumarin 3,4-epoxide and ortho-HPA products from the epoxidation pathway. Most of the studies on CYP2A6 polymorphisms are published after the 2000 IARC review and can help us identify vulnerable groups within each population.

In addition to findings on human CYP2A6 polymorphisms, a number of clinical trials and case reports indicates that coumarin causes hepatotoxicity in susceptible individuals. There are also new findings from toxicogenomic studies identifying several common cancer-related pathways altered by coumarin in both rat liver in vivo and human hepatocytes in vitro.

With this, we conclude our presentation today.

Thank you.

CHAIRPERSON MACK: Thank you, guys. That's very interesting -- a very interesting presentation.

Now, let's see if the Committee has any questions for the staff?

David

COMMITTEE MEMBER EASTMOND: I have a couple of questions. First of all, thank you for the presentation. And I'm encouraged to see that you're using some of this toxicogenomic data. Although, I realize it's a challenge

to sort of interpret and that's the way I approach it.

But I am curious, in this toxicogenomic data, typically they're looking at changes in gene expression, correct? So when they're showing evidence of nucleotide binding, this isn't reactive species binding to DNA like we think about in toxicology. This is nucleotide binding as far as gene expression changes, is that correct?

DR. HSIEH: That's correct.

COMMITTEE MEMBER EASTMOND: So this really isn't evidence of electrophilic species at all, and probably not an evidence of genotoxicity either. It's just saying it changes gene expression. I mean, that's my interpretation. I haven't looked at the data, but that's -- the sort of things that are picked up in a gene expression profile wouldn't tell you if it's electrophilic or anything like that, is that correct?

DR. SANDY: Well, David, what we explained was the analysis is you're using GO and KEGG pathway analysis. And they're linking changes in genes to different biological processes or pathways. So these -- they saw genes linked to pathways associated with the cellular response to nucleotide binding that were changed. That's what this is.

COMMITTEE MEMBER EASTMOND: Yeah, but that --

DR. SANDY: You're correct. It's not --

COMMITTEE MEMBER EASTMOND: -- that's not usually the way we think of it.

DR. SANDY: -- a apical endpoint, we measured nucleotide binding, no. It's looking at genes associated with certain pathways and processes.

COMMITTEE MEMBER EASTMOND: But I think this is the difference between sort of a how a molecular biologist would look at things, and how a toxicologist would look at things. Nucleotide binding usually refers to binding that alters gene expression versus where we think of as toxicologists, it usually refers to covalent binding, you know, to DNA or RNA.

So I think it's -- for me, I mean, I think it's certainly fine and reasonable. I don't -- I'm not necessarily convinced that's evidence of electrophilic properties of the compound.

CHAIRPERSON MACK: Anybody else?

Joe.

COMMITTEE MEMBER LANDOLPH: Sorry. Was the coumarin 3,4-epoxide mutagenic strongly so and dose-dependent mutagenesis in some of the systems studied?

DR. HSIEH: Dose response, yeah.

DR. SANDY: No. You're asking if the 3,4-epoxide was ever tested, and no, it has not been.

DR. HSIEH: No. No, it hasn't. Yeah.

COMMITTEE MEMBER LANDOLPH: Has not been tested.
DR. SANDY: On that metabolite slide, we said that is one of the metabolites that wasn't tested.
COMMITTEE MEMBER LANDOLPH: And how about ortho-HPA, the aldehyde ring open metabolite?
DR. HSIEH: It hasn't been tested.
COMMITTEE MEMBER LANDOLPH: And have they tried to radiolabel them and see if they bound covalently to DNA --
DR. HSIEH: Yes.
COMMITTEE MEMBER LANDOLPH: -- and made adducts?
DR. SUN: They're bound to microsomal proteins, but haven't tested for DNA.
COMMITTEE MEMBER LANDOLPH: To proteins but not DNA?
DR. SUN: No.
COMMITTEE MEMBER LANDOLPH: Okay.
CHAIRPERSON MACK: Yeah.
COMMITTEE MEMBER DAIRKEE: I have a follow-up question on the micro-irradiator. How sustained are these changes? So I understand that some of this is in vivo data, where the animals were treated with coumarin and then their livers were collected and microarrays were done to examine gene expression. How sustained are these changes is my question? Have they done different time

points after treatment?

DR. HSIEH: Yeah. They do several different time points from 1 day up to 2 weeks, several different time points. But the data use for the KEGG and GO in our analysis is the data they collect after one-day treatment.

COMMITTEE MEMBER DAIRKEE: So it is a very short-term change -- epigenetic change?

DR. HSIEH: One day.

COMMITTEE MEMBER DAIRKEE: Within one day the early changes that happen include what's on the KEGG pathways.

DR. HSIEH: Yeah, that's the data I should. But in the paper, they did do the study of one day up to 28 days. Yeah.

CHAIRPERSON MACK: Yes. Peggy

COMMITTEE MEMBER REYNOLDS: So I also thank you for your extensive review. As an epidemiologist, I have a much simpler and more fundamental question. And so my understanding is most of the animal evidence is based on exposure via feed or gavage. And that we're talking about what -- it's not clear to me whether we're talking natural or synthetic coumarin. But in the IARC report, there's an extensive discussion of the use of coumarin in personal care products.

So my question is you talked about potential for

oral versus dermal exposure. Given that this is prevalent in personal care products still, I presume, and I presume we'll hear something from the Fragrance Association since they provided a very extensive comment on this. What is the opportunity for exposure pathways in humans, and do you have any sort of sense of that in terms of pathways of exposure for potential risk.

DR. SANDY: Well, so -- again, your task is hazard identification. And that's what our document focuses on not exposure assessment. But as we discussed in the document, it is present in foods, naturally. It occurs naturally in some foods and it's used whether -- it's the same compound whether it's synthesized or extracted from a plant.

It's used in Perfumes, personal care products, and other things. So we would presume that the routes of exposure would be dermal and inhalation a oral.

COMMITTEE MEMBER REYNOLDS: Thank you.

CHAIRPERSON MACK: Yes Dave.

COMMITTEE MEMBER EASTMOND: I have another question. This has to do with, for example, the kidney tumor data -- incidence data that was seen by -- in the studies done by the National Toxicology Program. This would be on like slide number 7, slide number 9.

You have different denominators there for the

animals that were studied. And I'm -- I would guess that in some you're looking at total number of animals, and in others you're looking at the number of animals survived beyond a certain period. This strikes me a little bit of sort of cherry picking data, in that at least with the kidney tumor incidence, number 9 -- before this.

So on this one, if I recall NTP did not consider this statistically significant in the trend test -- their trend test, because they worked with the 50 animals per dosage, but apparently you've worked only on -- those that survived beyond a certain period of time.

If you go to slide number 7, its even more apparent. So the data for the adenomas you've got 55 animals, but for carcinomas you have 37 animals in the controls. And the fact that you're using different numbers of animals in your denominator strikes me as unusual for the same study.

DR. OSBORNE: So we have a standard way of calculating the denominator, where we look at the day of the first tumor -- the first tumor was seen and then we count how many animals were a live at that point. And so, we do that for each of these -- for each of the tumor types here.

COMMITTEE MEMBER EASTMOND: So you don't do -- so you do it for each tumor type and not in general, because

then that seems odd to me, because you've got 55 animals for adenomas, but you only have 37 animals in the control for carcinomas and vice versa all the way through the thing.

DR. OSBORNE: Yeah. Well, want to make sure it's -- that all the animals lived until a certain time point when they had their chance to develop the first tumor.

DR. SANDY: Let me add. This is a standard way that U.S. EPA uses as well when they have the information. So with NTP studies, we have all the information. We know the exact day that an animal died and was assessed with a tumor. And the -- the -- so we do this for dose response. We do it for hazard identification. We've been doing it for eons, years, every document you see where we have the data.

So for the particular tumor type, in this case, renal tubule carcinoma, it's the first day that any animal in any of the groups, controls or treated, was found to have a renal tumor -- tubular carcinoma. We say that's -- any animal that lived up to that day was then at risk of getting that tumor. If they died before that first tumor was seen, they're not at risk, so we don't count it in the effective number.

And so you can tell that for renal tubular

carcinomas, the numbers are lower. That means the carcinoma appeared later, in a later day. So there were fewer animals alive in all the groups. The renal tubular adenoma occurred earlier, apparently. And we have the day of occurrence in our HID, I'm sure.

And then when you combine the 2 tumor types, you're taking any animal that either had an adenoma or carcinoma, so based on the first day of the -- of either one of those tumors. So the denominators in that combined row are equivalent to in the adenoma row.

COMMITTEE MEMBER EASTMOND: So let me -- on some of the carcinoma data, it's pretty apparent, because there's only one animal that had a carcinoma. So you're saying when that animal developed a carcinoma at the 25 milligram per kilogram dose, that there were 37 animals alive at that time in the controls 35, 25, 19, and 13?

DR. SANDY: (Nods head.)

COMMITTEE MEMBER EASTMOND: And presumably, you didn't do this with the Carlton et al. study, because in that case, you didn't have the data, because there's massive mortality in those studies.

DR. SANDY: They didn't report the data. And when we -- when they don't report the data, we have to use the number of animals in the groups to start with. That's correct.

COMMITTEE MEMBER EASTMOND: All right,

CHAIRPERSON MACK: A couple of much less sophisticated questions. Dr. Hsieh, when you were reading through the literature, did you come across any source of coumarin that was extensively used in Southeast Asia or in South China? Cassia is the only one I can think of, which is cinnamon -- it's called Chinese cinnamon, which is actually now a more Mexican cinnamon, but that's -- but there's nothing else, I guess, because tonka beans are south American not Asian.

So as far as you know, there's no real extensive use of coumarin -- of plants which contain coumarin in Southeast Asia or South China, right?

DR. HSIEH: Coumarin also contained in lot of -- in a lot of personal care products, cosmetic and perfume. So like most perfume, 80 percent of perfume contain coumarin.

CHAIRPERSON MACK: I can't understand.

DR. HSIEH: Personal products.

CHAIRPERSON MACK: I'll get her to repeat what you said, because I'm really deaf.

DIRECTOR ZEISE: Okay. So lots of perfumes contain coumarin.

DR. HSIEH: Yeah.

DIRECTOR ZEISE: And I think Martha can add in

terms of foods in Southeast Asia.

CHAIRPERSON MACK: Yeah, I was really asking about food. I understand about perfumes.

DR. SANDY: And I can say that -- I can't answer to Asia or Southeast Asia, but there are so -- there are many other plants, lavender, and -- it's in our documents --

CHAIRPERSON MACK: Yeah, right.

DR. SANDY: -- Woodruff -- and a lot of other sources of coumarin in natural plants. So I can't tell you specifically for that part of the world.

CHAIRPERSON MACK: Okay. Thank you.

Second question is for Dr. Osborne. I'm -- I find the distinction between metastasizing and non-metastasizing cholangiocarcinoma to be kind of an odd distinction, because if you kill a mice at 2 years, you may not have found -- given them enough time. So I don't think that's a real distinction or it may well not be a real distinction. It may simply be a matter of how rapidly it metastasizes. So that's one -- an observation question.

So that seems reasonable to you?

DR. OSBORNE: Yeah. We reported -- this is how the authors reported it, so we didn't have the separate data for metastasizing or not.

CHAIRPERSON MACK: Okay. The next question is I don't know what an oncocytoma is?

DR. OSBORNE: Oncocytoma?

CHAIRPERSON MACK: It sounds like a cancer of the cell, but I have no idea what it means. Do you know what the cell of origin of an oncocytoma is?

DR. OSBORNE: Yeah, it's a renal tubule tumor.

DR. SANDY: It's also -- the cell of origin is the renal tubular cells. It's a very uncommon tumor type in NTP studies.

CHAIRPERSON MACK: So it's referred to other an unknown.

DR. SANDY: Pardon me?

CHAIRPERSON MACK: Other unknown?

DR. SANDY: It's uncommon in NTP studies, and it's actually the first time I think we've seen one reported.

CHAIRPERSON MACK: Okay. The final question is I'm really interested in the gene environment interaction that CYP26C -- whatever it is, 264 produces. And my question is does this distinction -- is there any evidence that this distinction makes a big difference between a urinary exposure of the metabolites or hepatic -- in other words, does it get into the bile? You pointed out that 1.5 percent, a very small percentage, gets excreted in the

bile. And yet, there's a lot of hepatotoxicity.

And so I would wonder if the -- if the CYP enzyme might make a difference in how it's distributed in discretion. Is there any evidence of that?

DR. SUN: From what I have would seen, I think in the presence or absence of CYP2A6 polymorphic -- polymorphisms, the urinary excretion remains the major metabolism pathway.

CHAIRPERSON MACK: But as far as you know, the hepatic excretion doesn't very much. I understand that urinary excretion is going to be the vast majority, but 1.5 percent is very small. And if it doubles or tripled, it might be important. No. No, evidence of that.

DR. SANDY: I don't think anyone has looked. That's the problem.

CHAIRPERSON MACK: Okay. That's all my questions.

COMMITTEE MEMBER ZHANG: I have a very simple question, just try to clarify a couple of slides.

Slide number 24. So CYP2A6, I know the -- on the slide 25, the next slide, is focused the Zhou 2017. So that's all from that study. But my question is the slides before, slide 24. Is that the slides already included the information in the Zhou 2017 or not? Because I know you found the other knew study later, so that's -- clarify.

DR. SUN: Yeah. Information from this slide, slide number 24, comes from the HID table 17. We just represent it in a graphic form instead of in a tabular form in the HID.

COMMITTEE MEMBER ZHANG: Okay.

DR. SUN: So the frequency in this figure comes from groups of studies that OEHHA reviewed.

COMMITTEE MEMBER ZHANG: Including Zhou 2017 or not?

DR. SUN: Not including Zhou.

COMMITTEE MEMBER ZHANG: Okay. That's my question. Number 2 question, also just clarifying the slide number 30 is about OEHHA's GO KEGG pathway analysis. Is this table, the data come only from the one study from the -- what are called the --

DR. HSIEH: Uehara, et al., 2000 --

COMMITTEE MEMBER ZHANG: 2008?

DR. HSIEH: Yeah, yeah.

COMMITTEE MEMBER ZHANG: So that's only from that one study, right?

DR. HSIEH: Yes.

COMMITTEE MEMBER ZHANG: Thank you.

I'm actually very glad that OEHHA this time at least they're to using the comparative toxicogenomic database trying to provide some additional information.

But I would save my comments on that later when we get there, but thank you.

CHAIRPERSON MACK: Anybody else?

Okay. I'm asking for public comments. And Jay is the only person who has provided a card. So, Jay, would you like to give us your five minute presentation?

DR. MURRAY: Thank you.

I'm Dr. Jay Murray, and speaking oh behalf of the International Fragrance Association, North America, which submitted written comments to you on coumarin.

(Thereupon an overhead presentation was presented as follows.)

DR. MURRAY: So -- and thank you for reading this submission as well as all the other documents you had to read. So coumarin is before you today, because no authority authoritative body has formally identified it as causing cancer. In fact, an authoritative body, NTP, conducted one of the cancer bioassays in animals, as you've seen. But NTP did not find enough evidence of carcinogenicity for coumarin to be listed under the authoritative bodies mechanism based on NTP's interpretation of its own bioassay.

No epidemiologic studies of coumarin in cancer have been identified, so it really comes down to the animal studies.

So if I could have the first -- my first -- you've got it up there. First and only slide.

There are 2 key cancer studies in animals, as you've already heard. It's the NTP and the Carlton studies. And they evaluated coumarin in mice and rats, both of them. NTP concluded that there was clear evidence of carcinogenic activity in female mice, but not in male mice or in male or female rats.

The clear evidence in female mice is due primarily to statistically significant increases in lung adenomas and carcinomas at the high dose, which is a tumor of questionable relevance to humans.

In male mice, there was an increase in benign, but not malignant, lung tumors at the high dose. And this is important, because your guidance criteria says the evidence must clearly show the chemical causes quote, "invasive cancer in animals", unquote.

In male rats there was an increase in benign renal tumors without a clear dose-response relationship, and in female rats no increase in tumors at any dose. So switching to the Carlton study, there was an increase in liver tumors in female mice at the low dose only. And according to IARC, no statistically significant increase in tumors in male mice, when adjusted for mortality.

In rats, there was no increase in tumors except

at the high dose, which greatly exceeded the maximum tolerated dose. So, for example, during the first 13 weeks of the study, the high dose male and female rats gained 266 and 102 grams less weight respectively than the control males and females. By the end of the study, males and females weighed 252 and 229 grams less, respectively, than the controls.

For those who may not do animal studies, those are massive differences in body weights. And in this day and age, the high dose would have been terminated because it drastically exceeded the maximum tolerated dose, and out of concern for animal welfare. You wouldn't see this these days.

The high dose should not be considered scientifically valid testing, according to generally accepted principles.

Now, IARC evaluated these studies and concluded, as you heard, coumarin could not be classified as a carcinogen, Group 3.

The HID also included information on CYP2A6 polymorphisms and genomics data which you heard today. I'm a fan of genomics data myself, and have been involved in several studies now looking at genomics data, but it provides little additional information of value for hazard identification.

Looking at genes where you see upregulation in glutathione metabolism or oxidative stress, that's not unique to chemicals that cause cancer. It may be a key characteristic, but you see it in lots of chemicals that don't cause cancer.

Regarding genotoxicity, the weight of the evidence does not show coumarin is genotoxic, you just heard that coumarin was negative in every single in vivo genotoxicity study. It was shown not to bind DNA in liver and kidney in Sprague-Dawley and F344 rats.

So, in conclusion, the only clear evidence of carcinogenicity is the increased incidence of alveolar/bronchiolar adenomas and carcinomas among high dose female mice in the NTP bioassay.

Clear evidence of the carcinogenic effect in one sex, of one species, in one study is not enough to list coumarin. The overall scientific evidence does not support a conclusion that coumarin has been clearly shown to cause cancer.

Thank you. I'd be happy to try and answer ay questions you might have.

CHAIRPERSON MACK: Thank you. Jay. Is there anybody who has question for Jay?

COMMITTEE MEMBER REYNOLDS: Just a question -- well, first of all, of course, IARC's classification is

with regard to humans, class 3 classification

DR. MURRAY: Yes.

COMMITTEE MEMBER REYNOLDS: So if coumarin is in a personal care product, it's in my shampoo, is that included under the rubric of fragrance, so that is considered trade secret? So a consumer wouldn't know that it's in a particular product or how much?

DR. MURRAY: I don't know the answer to that. I listened to your question earlier, and while I'm here on behalf of the International Fragrance Association. I'm a toxicologist. I'm really not an expert in fragrances or perfumes.

And, Dr. Reynolds, to go back to your first comment, IARC looks at both the animal and the human evidence. And IARC can classify a chemical as a carcinogen on the basis of the animal studies.

So, you know, a 2B classification. So they looked at these animals studies and said not enough to classify it and gave it a group 3.

COMMITTEE MEMBER REYNOLDS: Okay. Thank you.

CHAIRPERSON MACK: Okay. Thanks, Jay.

COMMITTEE MEMBER EASTMOND: Just a clarification. I think IARC considered the evidence limited in animals is the way they concluded.

COMMITTEE MEMBER REYNOLDS: Yes. There's limited

evidence in experimental animals is what it says.

CHAIRPERSON MACK: Anybody else have questions for Jay?

Okay. Thanks.

DR. MURRAY: Thank you.

CHAIRPERSON MACK: Okay. So now we have come to comments from the Committee. So I'd like to take it in the following order. I'd like to hear there Joe and then from David and then from Dr. Zhang, and then anybody else that wants to weigh-in.

So, Joe.

COMMITTEE MEMBER LANDOLPH: Okay. Jay, I did read your comments. I read all the public comments first before I looked at the HID. This is an interesting compound. You know, it's metabolized by cytochrome P450. You've got to two potential proximate carcinogens. One is the 3,4-epoxide, the other one is the ortho-HPA. So that was interesting, and gives a lot of insight into what the compound is doing.

I looked through the same database that Jay just discussed, and I have a little bit of a different take on it. In, let's see, table 3, the kidney data was interesting for the renal tubule adenomas, the tumors go from 1 to 6 to 8, and then to 5. And I think that's an increase in dose response, which then plateaus out.

The renal tubule carcinomas are not so robust. The combination of the two together again goes from 1 to 6 to 8 to 5. And the trend is not significant, but there are increases in tumors there over the control. I'm going to skip through some of this real quick. I also looked at the cholangiocarcinomas were interesting and in particular the non-metastasizing ones.

They go 0 at the control to 0 to 0 to 0, then to 1, and then to 31, and that trend test is statistically significant at P less than 0.001. And the high dose is 31 tumors out of 65, and that's statistically significant too.

Then the hepatocellular adenoma and carcinoma combined in the male Sprague-Dawley rats starts out a 2, in the controls and then it goes 2, 1, 1, then 6 - so that's an increase - then 29. So -- and that 29 is statistically significant and the trend is statistically significant.

Then the liver tumor incidence in Sprague-Dawley female rats shows a similar thing with the non-metastasizing cholangiocarcinomas going 0 in a controlled, 0, 0, 0, 0, 22 out of 65. That high dose event is statistically significant. The trend is statistically significant.

And then hepatocellular adenoma or carcinoma goes

0 in the controls to 0, 0, 0, then 1 - so it's going up a little bit - then 12 out of 65. The high dose end is statistically significant. The trend is statistically significant. So I added that data.

Then the male B6 -- C -- 6C3F1 mouse data is interesting too. There's a high background in the controls for the alveolar or bronchiolar carcinoma. It goes from 14, and then it drops to 8, then 14, then 24 out of 45 at the high dose. The trend is statistically significant. P equals 0.001. And alveolar/bronchiolar carcinomas is very low, not statistically significant for trend test. The combined goes from 14 to 9 to 15 to 24.

So it has a marginal increase at the next to the highest dose and a high increase 24 -- 25 tumors out of 45 mice. The trend is statistically significant. The high dose is statistically significant.

And I could go on, but to make a long story short, I'm going to say that I look at this data, and when I valuate the genotoxicity database, yes, a lot of that is in vitro work. It's a little bit tougher sometimes to get in vivo positive genotoxicity data. It's not so easy, but there is positive data there. So when I add this data together in my mind, I would say I certainly could not ignore this data. It's too much. It's positive at a number of doses, and trends are positive in some areas.

So my recommendation is to list this compound as a carcinogen.

CHAIRPERSON MACK: Thank you, Joe.

David.

COMMITTEE MEMBER EASTMOND: Thanks. I have a little bit different opinion, Joe, on some of these things. Let me just talk a little bit about how I approached this.

Initially, when I read through the document, I thought, wow, this was overwhelming evidence. But then as I started reviewing in more detail and some of this and looking at other authoritative bodies, I realized this is actually a really challenging compound, because the evidence is not nearly as compelling as what it might appear at first glance.

And so let me talk through some of these studies and put it in a context. So on the rat -- let's say, the kidney tumors in the rats in the NTP bioassay in the male rats, essentially, you know, these are described as rare tumors. I don't like that description, because you have tumors seen in the controls. So actually "uncommon", I think is a better descriptor of this particular tumor type. And that happens over and over again.

But, in essence, these tumors there's again a non-significant trend with a significant increase seen at

one of the intermediate doses. This is occurring in the context that these animals all have sort of chronic nephropathy. They're a hundred percent of the animals, I believe.

And so there is a real debate in the toxicology community on the significance of renal tumors when you have this chronic nephropathy in rats that's seen. So this -- put this -- and this happens for the males and the females. So you've got -- it's sort of questionable. The NTP considered this to be some evidence of carcinogenicity. And I would probably agree, there's some evidence there, but it becomes a challenge in sort of the interpretation. If we go onto the rat study, the Carlton studies -- and I'm usually not one to dismiss studies of having exceeded the MTD, the maximum tolerated dose, part of that becomes because of the way that maximum tolerated dose is actually defined in its origin.

But I think it is clear when you get a study where the dose is sufficiently high that basically the animals are under tremendous physiological stress, in some respects. In this case, they are -- I mean, the body weight -- and, indeed, the whole study has some serious problems, because in the female arm of this study, I think the survival in the controls was 26 percent by the end of the study.

So it's not -- you know, the study is a real problem. And on the rat part of the Carlton study, IARC reviewed this and considered it inadequate to use for doing an evaluation. So they kind of dismissed it. They did indicate -- it was kind of peculiar. Actually, I found it very odd that they dismissed one of the trends based on mortality-adjusted statistical analysis that they were aware of, that industry had conducted, but they apparently didn't see it, which was a very odd thing for them to do, in my mind, but that's a different thing.

So again, all the -- all the tumors, and there are very high tumor incidence in these bile duct tumors in the rats, but it's at the highest dose only. And that's sort of this what you could easily argue had exceeded a maximum tolerated dose.

Personally, I didn't consider the rat portion of this to be scientific valid. There -- the survival is such, and the body weight changes are such that I would not put weight on the rat portion of this study. And that's really basically both on the males and females portion of it.

The other rat studies were old studies, as they indicated, were not adequate for making determination. So if we go down into the other -- looking at the mouse now, we have the real strong clear evidence of carcinogenicity

in the female mice for lung tumors, 13-fold increase, a very strong response.

And so that's these strongest evidence as well. The second piece of evidence is in the female mice. There's an increase in liver tumors. And although, the trend is not statistically significant, the 2 intermediate doses have high values of liver tumors, and they're well outside of the NTP historical control.

So the NTP considered this as one of the reasons for considering that tumor caused cancer in the -- these female mice, because of this high, high level. So that's the clear evidence on those two different tissue types.

The liver could go some to clear, but I think because it's so far above historical controls, I'd consider that to be real evidence.

When we get into the forestomach tumors, again, NTP concluded these may be treatment related, but they -- so I would consider this sort of limited evidence. The Carlton one, you have a 2 times 2-fold dose relating to increase in lung carcinomas seen at the highest doses in the mice now.

And in this case, the body weight changes were not terrible. There was an 18 percent decrease in body weight gain in the highest dose male mice. And again, this was discounted by IARC because unpublished

mortality-adjusted data.

In the female mice, again, there's a significant trend seen at low dose. The author has indicated that this was within the historical control values, but they didn't provide any data. So it's harder to evaluate that.

As I said, the hamster study was inadequate, and there was a study in baboons, pretty unusual, but it was an unusual -- inadequate length. The length was not sufficient.

So you've got kind of a mixed pattern there. Let me move on and talk a bit about genotoxicity, because this is an important component. I found the genotoxicity data, the in vitro, just in general summary there's some evidence that coumarin causes genotoxicity in vitro, but it's a very weak in vitro genotoxin.

If you look at the result in TA100 in the rat liver S9 induced, it's under a 2-fold increase, which generally is sort of -- a rule of thumb in industry, if you don't have more than a 2-fold increase in TA100, that's not considered significant.

But NTP considered one trial to be equivocal. The other one they had a 1.9-fold increase, so they considered that to be, you know, evidence of -- that was mutagenic.

The other studies -- the 2 other supportive

studies are both abstracts, so you don't have the data there. And then the third one is in a substrain of TA100, in which most of the substrain was negative, but they did have a positive result, and they didn't describe the magnitude of the increase.

So there's a weak increase in say TA100, it's on mutation and bacteria. There's no increase in mutation see mammalian cells. If you look at the evidence on chromosomal aberrations in vitro, it's again that it's very weak. In fact, the concentrations, if you've figured out the maximum dose for testing currently by OECD standards is 10 millimolar. The effect is seen at 11 millimolar, so it's outside of the range you would test normally. That's the maximum test range. For that -- and that's a weak increase as well.

One of the earlier studies by Sanyal et al., I don't know how they got statistical significance. They essentially have a 30 percent increase in micronuclei above control. And they used the Kruskal–Wallis test and they don't have enough replicates in my mind to pick up significance. So I don't know how they did that. So, I mean, there is an increase there, but it's sort of suggestive.

So down the line, if you get into the old chromosome damage in the plant cells, these are studies

from the 1940s and 1950s, there's no data presented. The quality is the really marginal. In some cases, they essentially say, rarely chromosome fragments were seen, and that's what's the basis for, you know, the positive results.

So, for me, the in vitro stuff is -- yeah, there's some evidence genotoxic in vitro, but it's a pretty weak genotoxin. When you go in vivo, it doesn't appear there's any evidence for essentially mutagenicity and drosophila or chromosomal damage in the bone marrow cells and peripheral blood cells. In mice, negative in the UDS assay, which is a little unusual.

If you think this is causing point mutations in the liver, UDS assay is basically a very insensitive assay, but where it should pick up things or things that cause point mutations in the liver of the target organ, and they didn't pick up anything.

And the other thing is that it was negative in -- for covalent binding in the liver with -- and the kidney in both Sprague-Dawley and Fischer 344 rats. So there's no evidence for genotoxicity in vivo for this.

Moving on briefly to the metabolism work. It's correct that -- I mean, I would agree that there's two different basic metabolic pathways, one predominance. It's the one where you have the epoxide form in the

rodents. That is a very minor -- relatively minor pathway in humans. But because there are people who are polymorphic for CYP2D6, I guess -- or 2A6. 2A6.

Anyway, for this 2A6, there's a percentage of humans probably roughly five percent that would not metabolize during -- through the one pathway and they would go most likely through the epoxide pathway.

So I can't -- you can't conclude that this is only seen in rodents, and there's evidence in human clinical trials of liver toxicity as well.

So that's a long -- and the other thing I should say is that EFSA did review this. They considered the evidence sufficient. I think they considered that evidence of carcinogenicity is sufficient in rats, and -- but with supportive evidence in mice. I would probably flip that around, in my evaluation.

So I guess the bottom line on me is this is really a judgment call. I tend to think that we've got clear evidence in the female mouse both in the lung and the liver. I think there's enough evidence when you start looking around sort of the overall pattern that there's probably sufficient evidence to list.

However, in my recommendation, I would request that OEHHA really sincerely look at this as an -- for evidence of genotoxicity, because I do not think this

is -- would be considered a genotoxic carcinogen. And that was the conclusion of EFSA. They've gone through like 4 reviews of this, and that's their conclusion. It's a non-genotoxic carcinogen.

So that's my sort of summary. I can answer questions if you have them.

CHAIRPERSON MACK: So you're on the offense, but falling off.

COMMITTEE MEMBER EASTMOND: Yeah, I'd probably lean towards listing but just barely. I could go either way. And the one think I forgot to say on the genomics evidence, and Jay kind of summarized this is that the 2 pathways that seem to flag are glutathione changes and reactive oxygen species. And that's common for other chemicals that we haven't listed. Acetaminophen. If you're looking to acetaminophen, it would show those same patterns almost for sure.

So that for itself is not sufficient evidence for me to push it over the edge.

And the one last thing is all the animal studies we looked at were the same ones as looked at by IARC and EFSA, I believe too.

Thanks

CHAIRPERSON MACK: Dr. Zhang.

COMMITTEE MEMBER ZHANG: As Joe indicated, this

coumarin it is a very interesting compound, when I first reviewed the OEHHA documents. So to me I feel the animal data looks to me is pretty strong. You know, multiple species, multiple cancers, you know -- I mean multiple different organs. And the dose response same as, you know, observed in quite a few studies.

But what really bothers me is the genotoxicity data. It seems most of the genotoxicity data from in vitro, that's positive, and in vivo, you know, either not tested or negative, so that bothers me.

But as what I just -- you know, when I was asking OEHHA's staff for the questions, but what I'm really glad to see is I think this document the staff really took the trouble to really review each of the toxicogenomic studies one by one, and listed all that. And then use, chose the one of the study, and they did their own the comparative toxicogenomic database analysis, so I'm really happy to see that.

As what my Chair assigned me for the major role is trying to -- leading the discussion on the toxicogenomic data. And you already see a couple for -- our members raised these questions. So could I ask my Chair, so last night, I did some -- a little extra work. So I actually have a few slides, if I can --

CHAIRPERSON MACK: Go ahead.

COMMITTEE MEMBER ZHANG: -- before I could be allowed to present here. I could -- just a few slides, and who am I giving the data to?

Okay. Sorry.

So when she's loading the slides, I could tell you just a little story how could I get there?

I was not really intentioned to do this. Okay. Everything is by accident.

It's just the one PowerPoint presentation. It has my name at the end. Okay.

So when -- actually, last week I just came back from Lyon, France for IARC, yeah, working group meeting. So right after I come back with all the jet lag we had from that meeting.

So in that meeting I should have presented by our new post doc. I mean, is that, okay? I'm just telling a little bit of story how did I get to this.

So the new post doc named Linda, and they present -- so she actually has bioinformatic background. And they presented a study we're trying to using the existing database or software tried to see how could we apply this 10 key characteristics trying to predict any chemicals, especially unknown carcinogenicity chemicals if we can predict by using this 10 key characteristics.

So she present the data to the 2 IARC known, like

Group 1 carcinogens, which we are very interested. And randomly, she also chooses two Group 3 from IARC -- on the IARC list chemicals. So one of them she has no clue about the coumarin. I'm on this committee. But it's just the way she gave a presentation. She has very nice -- by the way, I also trying -- besides the known carcinogens were interested, I did two non-carcinogenic is really listed on the IARC Group 3 is not, you know, carcinogenic. So one of them is coumarin.

I didn't realize until two days ago she actually did that. So then when I was looking, oh, she already did coumarin. So yesterday, I was asking could you send me your slides -- she says what do you have done?

So I thought -- then I just get it yesterday afternoon. And then last night, I'm trying to take a look. I thought it would be good to just show a few slides.

So last night I'm trying to combine all what she did and summarize in a few slide. So that's -- the reason I thought of this is, number one is it's totally independent from what OEHHA has done with the comparative toxicogenomics database CTD. Okay. So this is number one.

Number two, and what Linda did is this, it is considered as lung carcinogen because it's from the Group

3 from IARC listing, so -- and also, I look at the -- when I was review -- I review the documents before. I see what OEHHA did is one by one from toxicogenomic studies here. What I'm trying to do, and that's what we are trying to do, is combine everything. We're not only focused on the six studies, whatever the studies we can find from this CTD database.

Okay. So let's just start. So that's just the two -- few days ago we did that. I can -- I don't have control. Do I have control?

--o0o--

COMMITTEE MEMBER ZHANG: Okay. So what we're -- first, what is CDT? It's a public available database, and it is robust. And, you know, the database was really trying to help us to better understand the interactions between exposure to the chemicals your interest or -- and the link to the human disease.

So that's what this basically generally database -- uh-oh. Sorry -- to do -- okay. Here -- so firstly, is you find all the genes related with your chemical of interest. Then you find the genes related to what's the disease you're interested. Then from this CTD database -- is that okay, I just give you a little background and how we come up about the data -- and the chemical and then the disease association. So that's

basically what it is.

--o0o--

COMMITTEE MEMBER ZHANG: So what's the goal of this little one? We really trying is identify the genes and the pathways and the key characteristics from the coumarin exposure. So I'm going to walk you quick through with a workflow.

It's a three-step workflow. The first is get into the CTD database. You try to obtain all the genes first -- all the genes associated with the chemical of your interest. In today's case, it's coumarin. The second is try to obtain all the genes associated with disease of your interest. So today let's just focus on cancer. Okay. So that's a Y. And when you have this two set of genes, you want to see if they have interactions or not. If they have no interaction period, you don't have to do anything, right? So which means chemical X doesn't have any association with disease Y. But if there is the association, you first want to see what are the genes overlapped. So that's first step.

Second step when we're trying to now load these genes -- overlapped genes into another software called Cytoscape. So in Cytoscape, they have specific app called the ClueGO. And I know you're using the GO, but here in Cytoscape, they call it ClueGO. It means what is this

genes to tell us? What's the clue.

Okay. What is the clue we got from this gene thing? So in here, what they're telling us is, first, gave us a visualized gene and gene interaction network, so you can make sense of what is going on, why -- you know, just now gave me a list of genes and see what's the interaction.

And it wasn't this really trying to look into this cluster of genes in a functionally group the network. That's what they were that is software could tell us. In the way, it's actually identify the pathways, which really link the chemical induces desisting, you know, involved with the specific exposure, with enrichment analysis.

So the third step is getting to the specific pathways called YK pathway, which is really based on the biological process, and biological pathways, and to really -- this one could help us to identify the key characteristics of the human's carcinogen.

So that's the goal. Identify genes, pathways, and the key characteristics. So that's my basic intro. What do we got?

From -- so now, I'm not even talking about everything. So from the CTD, identify the total of 65 studies, and they involve with the -- any coumarin genes, you can see the species is human or mice study or rats,

but it's some studies from this lots of studies with coumarin screens, but it didn't even specify what the species is.

So a total of 65 studies we identified from the CTD. And -- oh, sorry. I'm looking -- my computer doesn't -- let me close it, so I don't get. Okay.

(Laughter.)

--o0o--

COMMITTEE MEMBER ZHANG: So second we look at the genes. So you see it from the -- from the table, right? So from 65 genes identify total of 2 congener 76 gene, but only 222 is unique genes, because some genes will overlap with different species. So there's 22 -- 222 genes that's related with coumarin, but a formula database, there are more than 3,000 genes related with neoplasm, so related about all cancer. How many of them overlap?

You can see 96 genes overlap. And that's about 43 percent of coumarin genes with related with any type cancer. So in this term of neoplasms include with cancers of liver, lung, kidney, you know, what -- and many more other types.

We also did separately with just the liver cancer. You can also do just liver cancer. But today, I'm only going to show you the data from cancer in general.

--o0o--

COMMITTEE MEMBER ZHANG: So second step is what are all those 96 genes overlap genes tell us. We put this 96 gene name into this pathway analysis by using ClueGO. So this is all the pathway each does represent each pathway. How many of them?

So there are 44 pathways identified. But when you look at the 44 total pathways. Actually, it's only 52 genes from the 96 overlapped genes really involved in the identified pathways. So which means another 44 genes, which are not really involved in any pathway. Okay. So at least now we see what's -- what are the big dots -- all the big dots means that have more genes involved in this pathway. So more that's maybe only one or two genes. So that's -- so you can see what making sense.

Next, what's the pathway? I don't mean you to have see.

--o0o--

COMMITTEE MEMBER ZHANG: So I'm just going to show you the top 20 pathways, you know, from the WikiPathway Analysis. Okay. So you can see the top one actually is just called NRF2, and 1 and 2 is all involved either NRF2 or NFE2 or L2. It's also an NRF2.

Okay. And also the oxidative stress pathway is also on the top of 5. Take a look. Sorry.

Okay. I should be fine. I'm just trying to show you that I just using oxidative stress. Okay. What we have is in the database 61 genes involved in oxidative stress. But a sixth gene was identified from the coumarin related gene.

Okay. So then let me just quickly show you what's the data come out. The -- all the green genes that 6 genes identified from the coumarin involved in oxidative stress, so -- and also, oxidative stress is one of the 10 key characteristics as number 5 in the -- in the list, which the table -- I think the document has it.

--o0o--

COMMITTEE MEMBER ZHANG: So then when we are just looking at all the 10 key characteristics and how many pathways involved with each cases. So here, you can see the oxidative stress is the number one actually is involved with 10 pathway -- 10 coumarin-related pathways.

Okay. And then the first one metabolic activation, but I think that's because mostly they are involved as P450, and NSR.

So if you want to have detail, each one they give you, you know, a table of the list of what genes are involved in which pathways. So I'm just showing here.

So what have we actually learned from this is you can see the red -- 2 red. That's pretty straightforward

and we really see oxidative stress, metabolic activation, like there you have crushing about. But actually, you know, from the data analysis actually it seems to show up mostly effective, but maybe it's different questions. Is only the genes involved with this? But actually you want to think about that.

COMMITTEE MEMBER EASTMOND: Mine was nucleotide binding, which --

COMMITTEE MEMBER ZHANG: Right. Right. There's a different

COMMITTEE MEMBER EASTMOND: -- you have nothing for genotoxicity coming up, right?

COMMITTEE MEMBER ZHANG: Yes. Okay. We can go through. Why -- okay. That's good question. Why genotoxicity pathways is zero. If you think, most of the genotoxicity data coming from chromosome aberration, micronuclei, comet assay, you know, or mutations, right. Except for the mutations, you may have identified specific genes. Other things do not involve with specific genes. For this database is all based on specific genes has been tested with coumarin exposure.

So that's why the genotoxicity pathway is not shown here, unless we have specific -- the genes, you know, like mutation, which would have been involved in the database. That's my best explanation. We can discuss

about that.

I almost finished, then we can come back to this.

So what we got here is we see 5 key -- or the red and blue 5 key characteristics are involved in the potential carcinogenicity of coumarin. So that's basically...

--o0o--

COMMITTEE MEMBER ZHANG: Summarize up from the CDT database what do we have seen? Coumarin genes 2,222 -- and the cancer genes it 3,152, overlap genes are 96. From 96 genes, we're trying to -- you know, in there the 96 genes, we look at the pathways analysis. And what we see is 44 of genes they're not involved with any pathway, 52 yes, and then pathway involved with coumarin as the 44 pathways.

And then when we did the WikiPathways Analysis, but which I have to say, because we run out of time yesterday, we only did a very crude analysis about the key characteristics. So that's 5 out of 10. So we didn't have a chance to do the detail. So that's basically what I have got. Thank you so much.

I don't know if I made it in 10 minutes, but actually just tell you the whole story.

CHAIRPERSON MACK: Do you think we should list it or not?

(Laughter.)

COMMITTEE MEMBER ZHANG: What? Okay. So here --

(Laughter.)

COMMITTEE MEMBER ZHANG: Here is -- I have to say when I read what OEHHA provided me in the chapter 3.3.7 and 3.3.8, I feel on the fence.

(Laughter.)

COMMITTEE MEMBER ZHANG: Because it's each study, right. You have to think and you have to go in through -- issue I gave you different gene as to what's really making sense. So I'm actually glad we finally, you know, as really is by accident. You know, my lab, you know, post -- new post-doc did this, and which allowed me at the very last minutes -- actually, I had it last night working really hard to put this few slides together. And then after I did this myself, and I look at the general broad database, that make me more convinced that from genes pathways and the key characteristics from all 3 different I would vote to list. Is that what you want to ask me?

CHAIRPERSON MACK: Yes, that's fine.

Jason, do you have thinking to add?

COMMITTEE MEMBER ZHANG: Sorry, we're sharing. Good sharing

COMMITTEE MEMBER BUSH: I have a slide show as well.

(Laughter.)

COMMITTEE MEMBER BUSH: Teasing, teasing.

(Laughter.)

COMMITTEE MEMBER BUSH: So. So let me give you my interpretation of this. Thank you to OEHHA for compiling the HID document, and thank you to the Consumer Specialty Products Association, Council for Responsible Nutrition, and International Fragrance Association for submitting your comments.

I read and evaluated your concerns refuting the coumarin report. Like David, when I first looked at this, I thought it was clear. But as I dug into it, I found that the animal data, the multiple rodent studies across multiple tumor types, less -- less convincing.

One thing I found particularly disconcerting was the presence of the lung tumors, and, you know, possible extrapolation to -- you know, to use in tobacco products or vaping products.

But looking at the hepatocellular carcinomas, the cholangiosarcomas, I attributed that -- while the data was compelling, the CYP2A6 polymorphisms, I contributed that more to the cytotoxicity rather than carcinogenicity. And you know, cholangiosarcomas are derived from connective tissue in hepatobiliary area. And to me, that is more a result of an inflammatory response.

And it was interesting to see your interpretation, Luoping, having that chronic inflammation those -- that particular pathway.

COMMITTEE MEMBER ZHANG: Three pathways. Three pathways involved.

COMMITTEE MEMBER BUSH: Yeah. Right. Right.

You know, and while there is some clinical studies out there, I found showing the hepatotoxicity -- I think it's cytotoxicity. And, you know, any connection with clinical studies was more tenuous.

The genotoxicity observations, you know, I think were suggestive of DNA repair inhibition. But beyond that, I wasn't particularly convinced of any of the other mutational information.

I was also interested in the cell transformation information, but there was only limited studies there. I think one study on human fibroblasts showing marginal cell transformation with coumarin alone, and that only occurred at high dose.

In terms of the KEGG and GO pathway information. And I have quite a bit of experience with this on my own from my proteomic work. It is microarray data, a single study. And I think we have to be careful there. You know, often this -- this data can be misleading. And my interpretation was that it warrants further validation,

rather than, you know, making too much of that data.

So I find myself, you know, concurring with, you know, the other authoritative bodies that -- for me, the weight of the evidence, at this point, is too limited. And I would vote not to -- not to list at this time.

CHAIRPERSON MACK: Thank you.

Dr. Dairkee.

COMMITTEE MEMBER ZHANG: Could I ask a question? Sorry.

So, Dr. Bush, you -- I thought it is the mutation data is -- but my understanding I thought the mutation data they're pretty convincing at least from the Ames test in the -- in the, you know, the bacterial tests. They are repeated and consistently come up with this one specific target. And so do you -- you don't think the bacterial data counts?

COMMITTEE MEMBER BUSH: I put less weight on the Ames test than looking at eukaryotic cells. And for that, I wasn't convinced.

CHAIRPERSON MACK: Dr. Dairkee.

COMMITTEE MEMBER DAIRKEE: Yes. With all the comprehensive reading material we were given, I was not on the fence at all. It was very, very helpful, very clear to me as to how I feel about this -- this chemical. Especially looking at the mechanistic data, it is very

clear that the cytotoxicity, as Jason pointed out, goes along with the necrosis, the atrophy, the nephropathy. All of that seems to make so much sense, because that's what cytotoxicity does. It kills cells.

And because the evidence on cell proliferation is very inconsistent as well, in vitro, that the agent -- the chemical does not induce cell proliferation. In fact, it induces apoptosis. And when you look at all the genes that are going up, they are apoptosis genes. So it's not even inducing evasion of apoptosis, which is why -- or cell death, which is why -- and it's not causing cell proliferation. So obviously, you are having cell death going on.

And in vivo and the in vitro data are quite compatible. So even if there is some level of genotoxicity, if the cells are not able to survive past that, how are they going to make cancer? They cannot be cancerous.

So, in my opinion, the evidence really points to the fact that this may be a nasty chemical at high doses in terms of toxicity. But there's really no strong evidence mechanistically for carcinogenicity.

CHAIRPERSON MACK: Peggy.

COMMITTEE MEMBER REYNOLDS: Well, I just have to say as a mere epidemiologist, it was very helpful to hear

these discussions. I was primarily focused on the animal studies. And I felt like the evidence was extremely mixed and fragile. And so I was completely on the fence and did not fell strongly to list based on that.

CHAIRPERSON MACK: Okay. Well, I -- everybody is on the fence. And, of course, I am too, but I'm going to fall off. The thing that impressed me the most was the cholangiosarcomas, because even at very high doses it means that there's a potential for carcinogenicity.

I don't pay too much attention to the in vitro studies when that's true, because I don't know what the mechanisms is. But in the empirical piece of information from the rats at least, it causes carcinoma.

And our mandate, unlike that at IARC is not to decide for sure that it causes carcinoma in people, it's to whether it causes cancer. That's the way the wording is in the legislation. So I have to say that I think that that's real. And I'm motivated by something else, which may or may not be pertinent, but it sticks in my mind.

Cholangiocarcinoma is not a very common cancer in the United States. It's very rare, in fact. But there's one place where it is the single most common lethal cancer. And it's more lethal in that place than hepatocarcinoma, which ought to be the most lethal cancer. In Khon Kaen Province in Northeastern Thailand, this is

where cholangiocarcinoma is the most common cancer.

And the reason it is is not due to coumarin, as far as we know, and from what I'm told there's no reason to think there's any coumarin there. But both of my questions were related to this, because cholangiocarcinomas is a carcinoma of the -- in people and in rats as well, a carcinoma of those bile ducts that are within the liver, not after the liver, but within the liver.

And in Southeast Asia that carcinomas has caused quote unquote one of the causes of it is a parasite of fish that people eat raw in Northeastern Thailand and Laos. And the organism that the parasite is a fluke and the fluke lives in that -- those -- in those bile ducts within the liver for up to 20 years.

And the presumption is always that it causes cholangiocarcinoma by virtue of simple abrasion and injury to the cells of the bile duct. But we all know that for the most part, that's not enough to cause the cancer, at least it is for most kinds of carcinoma. So one always assumes there must be something else going on, and I don't know what it isn't, and I'm sure it's not coumarin. But cholangiocarcinoma is an important carcinoma. And even if it's caused in rats by very high doses, to me, it means that coumarin can cause cancer under some circumstances.

And I have to assume it probably can elsewhere as well.

So my vote is for listing.

So what do we do now?

Make a vote.

All right. So let me go to the right words to make sure I don't upset Carol.

Okay. The question is has coumarin been shown through scientifically valid testing, according to generally accepted principles, to cause cancer?

Now, may I have yes votes to that question by hand raises.

(Hands raised.)

CHAIRPERSON MACK: One, two, three. Three and a half, three and a half, going for three and three-quarters.

Four. Four out of -- so let's count again. Maybe I missed --

Only one, two, three, four.

Okay. The vote is not -- well let me just ask now the other question.

All those voting no, please raise their hand?

(Hands raised.)

CHAIRPERSON MACK: One, two.

Four yeses and two noes.

Five votes are required to add a chemical to the

list.

COMMITTEE MEMBER REYNOLDS: Abstain.

CHAIRPERSON MACK: And we have and abstention, but it's irrelevant.

CHAIRPERSON MACK: So we do not vote to list -- add coumarin to the list.

Did I count myself?

I must have, 4 to 2.

DIRECTOR ZEISE: There's 7 here.

CHAIRPERSON MACK: Okay. So we're finished with that particular item on the agenda, correct?

DIRECTOR ZEISE: Does the court reporter --

CHAIRPERSON MACK: Carol.

CHIEF COUNSEL MONAHAN CUMMINGS: I'm just thinking we need to take a break at least for the reporter. I know we've got other stuff and we'd like to go quickly, but I think we need to at least take a short break.

Could we do 15 minutes. Would that work for you?

THE COURT REPORTER: That's fine.

CHIEF COUNSEL MONAHAN CUMMINGS: You want to do that instead of taking a lunch break.

CHAIRPERSON MACK: How many minutes, 15 minutes?

CHIEF COUNSEL MONAHAN CUMMINGS: Uh-huh.

CHAIRPERSON MACK: Okay.

CHIEF COUNSEL MONAHAN CUMMINGS: Okay. Thank you.

(Off record: 12:09 p.m.)

(Thereupon a recess was taken.)

(On record: 12:21 p.m.)

CHAIRPERSON MACK: Okay. Can we reconvene, please?

Okay. Here.

DIRECTOR ZEISE: Yes.

CHAIRPERSON MACK: Okay. The next item is a consent item, in which the Committee is asked to consent to the update of the California Code of Regulations title 27, section 27000, the list of chemicals which have not been adequately tested as required. So this list is basically a list of chemicals which are both under question for both -- both carcinogenicity and -- I'm blocking on the word --

DIRECTOR ZEISE: Reproductive toxicity.

CHAIRPERSON MACK: Reproductive toxicity.

So the Committee just asked to give their consent to maintaining the same list. There are really only a couple of carcinogen potentials on the whole list. Carol, do you want to say something?

CHIEF COUNSEL MONAHAN CUMMINGS: Right. So as Dr. Mack said, this is a consent item. We're going to try

it this way at this meeting.

And so if -- let me see.

(Thereupon an overhead presentation was presented as follows.)

CHIEF COUNSEL MONAHAN CUMMINGS: If you recall, you received a document that looks something like this from us where it was a staff report that -- ahead of the meeting, and we also posted this report on our website that is shown in this slide. There's a copy available at the back of the room for the public if anybody wishes to see it.

The specific item you're voting on is amendments to -- that are shown in section 6 of that report. This item is on the agenda for your consent. This means you just need to vote yes or no concerning the changes OEHHA proposes to make to this Section 2700[SIC] list of chemicals that need further testing. And this is based on information obtained by OEHHA from the Department of Pesticide Regulation and U.S. EPA.

The section 2700[SIC] list is informational and has no regulatory effect.

Next slide.

--o0o--

CHIEF COUNSEL MONAHAN CUMMINGS: That's me. Next slide. Okay. So for purposes of this Committee, there's

only two changes to the list that are proposed in the staff report. You can see these on this slide. The other changes to the list will be considered by the DART IC Committee at their meeting later this month.

OEHHA staff is recommending that you vote yes, so that we can make the necessary changes to the list described in the staff report.

Does anyone have any questions before Chairman Mack requests a vote?

CHAIRPERSON MACK: Like the previous discussion, there doesn't seem to be any question at all, Carol.

CHIEF COUNSEL MONAHAN CUMMINGS: Okay. Good.

CHAIRPERSON MACK: So I go ahead and ask for the vote?

Based upon the recommendations of the OEHHA staff report should Section 27000 of Title 27 in the California Code of Regulations be amended as indicated in section 6 of the staff report?

Would everybody voting yes, please raise their hands?

(Hands raised.)

CHAIRPERSON MACK: Unanimously approved.

No votes for no, so the result is 6 votes yes, and no votes no.

So now we go on to the next item on the agenda.

CHIEF COUNSEL MONAHAN CUMMINGS: Sorry, that was 7 yes, 0 no.

DIRECTOR ZEISE: Because there's 7.

CHAIRPERSON MACK: You want to do that?

Are you going to do it?

CHAIRPERSON MACK: Basically, we've come to the staff updates. So we're talking about the Prop 65 chemicals that have been added since November.

I can read them, but I guess somebody else -- yeah, please. Go ahead, my dear.

MS. RAMIREZ: Okay. Since your last meeting, weve added a total of 5 chemicals administratively for causing cancer, and 4 for causing reproductive toxicity. The first slide here shows that for cancer the following chemicals were added: Glyphosate, by the Labor Code listing mechanism; Pentabromodiphenyl ether mixture [DE71 (technical grade)] by the authoritative bodies listing mechanism; and N,N-dimethylformamide; 2-mercaptobenzothiazole; and tetrabromobisphenol A by the Labor Code listing mechanism.

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MS. RAMIREZ: The second slide shows that for reproductive toxicity Vismodegib was added for all three endpoints, developmental, female reproductive, and male reproductive toxicity via the formally required listing

mechanism

Pertuzumab was added for the developmental endpoint also by the formally required listing mechanism.

And perfluorooctanoic acid, PFOA, and perfluorooctane sulfonate, PFOS, where both added for the developmental endpoint via the authority bodies mechanism.

--o0o--

MS. RAMIREZ: The next slide has the chemical under consideration for administrative listing, vinylidene chloride. The far right column indicates the date of the notice of intent to list. That was September 22nd, 2017.

--o0o--

MS. RAMIREZ: And this next slide shows that since your last meeting 8 safe harbor levels have been adopted in regulation effective July 1st, 2017. A no significant risk level has been adopted for styrene. And maximum allowable dose levels have been adopted for ethylene glycol (ingested), and for oral exposures to each of the 6 triazine compounds.

--o0o--

MS. RAMIREZ: On this last slide, as you can see, we've also proposed safe harbor levels for 3 chemicals. No significant risk levels have been proposed for malathion, glyphosate, and vinylidene chloride.

And now I'll turn things back over to Carol.

Thank you.

CHAIRPERSON MACK: Thank you. Now, we go on to litigation.

CHIEF COUNSEL MONAHAN CUMMINGS: Right. Litigation.

All right. So the good news since our last meeting is that there have been no new lawsuits filed against OEHHA. Now, there will be just because I said that, but there -- the existing cases -- active cases are all now in the court of appeal. The only trial court cases pending are derivative, and one that's not a Prop 65 case. We did settle the case Syngenta versus OEHHA that related to the no significant risk level for chlorothalonil, so that case has been dismissed.

And all the other cases have been fully briefed. We expect to hear, at some point, from the court of appeal for a hearing. They are the American Chemistry Council case that challenged the listing of BPA as a developmental toxin, also, the American Chemistry Council case challenging the listing of DINP, the Syngenta case challenging the listing of the triazines, a case filed by Mateel challenging our lead maximum allowable dose level, the challenge by Monsanto to the listing of glyphosate.

So all of those cases we expect, at some point, to be heard by the court of appeal. If I had to guess,

the most likely one to be heard early next year is the Monsanto case, because they have successfully requested a early hearing date on that case. We don't know exactly when it's going to get set. It's in the Fifth District Court.

So does anybody have questions?

CHAIRPERSON MACK: Is glyphosate Roundup.

CHIEF COUNSEL MONAHAN CUMMINGS: Glyphosate is in Roundup, yes.

CHAIRPERSON MACK: Any comments or questions for -- Dr. Landolph.

COMMITTEE MEMBER LANDOLPH: Usually, with the administrative listings, I usually look at them, and I usually agree with them because they've been so thought out already, so I don't say anything. But the last set, you know, they sent out, I agreed with them all. So I didn't say anything to you. Is that okay? Do you -- I think that's what most people do probably.

CHIEF COUNSEL MONAHAN CUMMINGS: Right. Well, it's our practice to send you notices when we do administrative listings. And you always have the option as individuals to comment on whether or not you think that that listing is appropriate under that particular listing mechanism, but you're not required to make a comment.

COMMITTEE MEMBER LANDOLPH: So you should assume

that if you don't hear from me, that means everything is okay.

CHIEF COUNSEL MONAHAN CUMMINGS: Correct.

COMMITTEE MEMBER LANDOLPH: If I don't like something, I'll let you know, but usually they're okay.

CHIEF COUNSEL MONAHAN CUMMINGS: Okay.

COMMITTEE MEMBER EASTMOND: Let me ask as a follow-up question. So what if we believe that listing was not correct. The problem is these authoritative bodies one are done pretty much automatically based upon sort of statute. So even if I didn't think something should be listed, what impact does that have in the decision-making process?

CHIEF COUNSEL MONAHAN CUMMINGS: Well, that's got a two-part answer. First, this Committee has identified authoritative bodies for purposes of listing carcinogens. So if, for some reason, you -- you noticed that a particular authoritative body is identifying chemicals that you don't think should be listed, then you always have the option to change that designation, and say they're no longer and authoritative body. That would have to be done by the Committee in, you know -- through a regular process.

If it's a listing under one of the other mechanisms, for example, the Labor Code or formally

required, you can make a comment as an individual on the Committee and say why you don't think that it should be listed. But then we'd still have to look at that in the context of the criteria in the regulation and the statute to see if it should still be listed.

COMMITTEE MEMBER EASTMOND: So I was surprised to see glyphosate was listed under Labor Code and not authoritative body. That was --

CHIEF COUNSEL MONAHAN CUMMINGS: Well, actually the International Agency for Research on Cancer is both an authoritative body and a source for listings under the Labor Code. And generally, we propose the listings through the Labor Code mechanism. Unless there's some confusion or something that needs to be fleshed out more in a public comment process, then we can put it through the authoritative body process.

So normally, we put them through the Labor Code, unless there's a -- there's a particular reason to put them through the other mechanism, but we can use either one for them.

COMMITTEE MEMBER LANDOLPH: So if a member of the CIC said they don't like this listing by authoritative bodies, we challenge it, then what would happen? Would OEHHA internally adjudicate that or would it come back to the Committee?

CHIEF COUNSEL MONAHAN CUMMINGS: Well, I think what we would do with any comments that you make on the proposed listings is consider them in light of the criteria for that listing mechanism for that particular chemical. So if you say, for example, you don't think it meets the criteria for listing, because it's not well identified, or it wasn't a final decision, or the science is not strong enough to support the decision by the authoritative body, then we would consider that in much the same way as we do other public comments.

But the other situation is where if you thought that a particular body was making decisions kind of routinely adverse to what you all would do, then you always have the opportunity to change the designation of your -- the authoritative body and not identify them anymore.

And in the alternative, you can also add authoritative bodies, which we really haven't done for many years.

COMMITTEE MEMBER LANDOLPH: Well, that would be pretty strin -- you know, pretty severe. I mean, occasionally they might make a mistake. Mistakes happen. So what if we thought it was a mistake, but they were generally a reasonable authoritative body, could we consider it by the Committee again?

CHIEF COUNSEL MONAHAN CUMMINGS: The chemical itself?

COMMITTEE MEMBER LANDOLPH: Yes.

CHIEF COUNSEL MONAHAN CUMMINGS: No, not generally. If it meets the criteria for listing in any of the four listing mechanisms, we have to list it. But like I said, if you have a concern about a listing, then I would encourage you to make those comments, so we can consider them while we're -- before we may finish the listing process.

For chem -- if a chemical gets to a certain point in the authoritative listing process, and we determine that maybe it doesn't meet the criteria anymore - we thought it did, but it doesn't - we will take that chemical to you for consideration before we decide whether or not to list it.

CHAIRPERSON MACK: You'll just have to write an op-ed. Okay?

(Laughter.)

COMMITTEE MEMBER LANDOLPH: Pardon?

CHAIRPERSON MACK: You'll just have to write an op-ed.

COMMITTEE MEMBER EASTMOND: I mean, I'll give you a case in point. A number of years ago when I was first on the Committee, we deliberated on trichloroacetic acid

at great length, and concluded that although tumors were induced in rodents, that they were not relevant to humans. A number of years later, it was listed through the authoritative bodies mechanism, or Labor Code, inde -- regardless of what we had concluded.

And you know that basically kind of undermines -- I find it sort of undermining the credibility of your Committee, if you think your Committee of experts has reviewed this, evaluated, and they've reached a conclusion, and then you list it regardless. It strikes me as not really following the recommendations or advice of the Committee.

CHIEF COUNSEL MONAHAN CUMMINGS: Well, I think that the -- the issue is the way that the statute is written. It has these independent listing mechanisms that aren't -- there's no hierarchy. So if -- as I said, if it meets one of those listing mechanisms, we have to list it.

And there's -- it's not that uncommon for there to be a difference of opinion between the different authoritative bodies or other groups. So I agree that it is uncomfortable. Sometimes, it's because there's newer evidence, but it's the way that the statute is written.

COMMITTEE MEMBER LANDOLPH: One more question. Sorry, one more question.

The chemical that's being considered on appeal,

it was one of those plasticizer chemicals, do we still have to hold on to documentation about that?

CHIEF COUNSEL MONAHAN CUMMINGS: The DINP?

COMMITTEE MEMBER LANDOLPH: Yeah.

CHIEF COUNSEL MONAHAN CUMMINGS: Yes, until the case is resolved. It's been sitting in the court of appeal now for probably close to 2 years, but it just hasn't been set for hearing.

COMMITTEE MEMBER LANDOLPH: Thank you.

COMMITTEE MEMBER EASTMOND: One quick question. A few years ago Governor Brown was trying to advance some changes in Prop 65 in the evaluation. Is he pursuing that at all, or has he kind of tabled that or stopped any efforts? Is that still moving forward?

CHIEF COUNSEL MONAHAN CUMMINGS: Well, there was an effort -- a pretty extensive effort to do some updates and modifications to the statute. As you may know, the -- it can only be changed by a two-thirds majority vote of the legislature, plus a finding that whatever change furthers the purpose of this statute. It's very difficult to get that. And he brought together a very large group of industry and NGOs, and a whole group of folks, including us, and we worked pretty hard to try and come up with something that would get through the legislature, but just ultimately weren't successful.

DIRECTOR ZEISE: You know, there was a -- coming out of that process also, we've changed the regulation governing how warnings are -- safe harbor warnings are given. And I wonder if at the next meeting, it would be helpful to -- for us to make a presentation to the Committee, because it does address some of the issues that came up in that process. So we can do that next meeting.

CHAIRPERSON MACK: Go ahead.

DIRECTOR ZEISE: Okay. So I'll summarize the Committee's actions.

The Committee considered whether or not coumarin had been clearly shown through scientifically valid testing, according to generally accepted principles to cause cancer. There were 4 votes for, 2 against, and 1 abstention. It requires 5 yes votes to add a chemical to the list, so coumarin won't be added to the Prop 65 list.

Then the Committee considered the Section 2700[SIC] additions and removals of chemicals requiring testing based on federal and State requirements. And the Committee considered that as a consent item. All Committee members present voted yes, so that amendment will be -- proceed through the regulatory process.

And so that's it for the Committee actions. And I just wanted to thank all the Committee members for again coming to the meeting, and spending so much time in

preparation of the meeting, all your careful consideration that went into -- I know -- we all know that you're so busy. So we really appreciate your input and donating your time to State service. So thank you.

And I'd like to thank the members of the public for your participation at the meeting, and for those listening on the webcast. And then, of course, the RCHAB and Implementation staff to put on these meetings and to prepare the hazard identification materials as you can see is a huge task. And the staff I think -- I've heard a number of compliments about the document, and -- that was produced for the hazard identification. So I just want to thank the staff again for all the work on that.

So now to you, Dr. Mack.

CHAIRPERSON MACK: Since they all work for Lauren, it's not -- that's a pretty shallow thank you. So I'm going to --

(Laughter.)

CHAIRPERSON MACK: -- thank you instead. You guys did a lot of work, and we really appreciated your doing it.

Thank you very much.

All right. I formally will adjourn the meeting now. Thank you very much.

(Thereupon the Carcinogen Identification

Committee adjourned at 12:41 p.m.)

C E R T I F I C A T E O F R E P O R T E R

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 16th day of November, 2017.

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