MEETING

STATE OF CALIFORNIA

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

PROPOSITION 65

CARCINOGEN IDENTIFICATION COMMITTEE

JOE SERNA JR.

CALEPA HEADQUARTERS BUILDING

1001 I STREET

COASTAL HEARING ROOM

SACRAMENTO, CALIFORNIA

WEDNESDAY, NOVEMBER 19, 2014

10:00 A.M.

TIFFANY C. KRAFT, CSR

CERTIFIED SHORTHAND REPORTER

LICENSE NUMBER 12277

A P P E A R A N C E S

COMMITTEE MEMBERS:

Thomas M. Mack, M.D., M.P.H., Chairperson

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, Deputy Director, Scientific Affairs

Dr. John Budroe, Section Chief, Air Toxicology and Risk Assessment Section

Ms. Carol Monahan-Cummings, Chief Counsel

Dr. Jennifer Hsieh

Dr. Kate Li

Dr. Gwendolyn Osborne

Dr. Karin Ricker

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

Dr. Feng Tsai

Dr. Patty Wong, Section Chief, Cancer Toxicology and Epidemiology Section

ALSO PRESENT:

Gordon Burns, Undersecretary, CalEPA

I N D E X

P R O C E E D I N G S

DEPUTY DIRECTOR ZEISE: Good morning, everyone. Let's get started. Hello. I'm Lauren Zeise. I'm Deputy Director for Scientific Affairs at the Office of Environmental Health Hazard Assessment. I'm sitting in for Dr. George Alexeeff, the Director, who wasn't able to make this meeting.

I'd like to welcome the Committee and the audience to the meeting, including those that might be listening via webcast.

The first thing I'll do is introduce the Committee. So the Chair of the Committee is at my left is Dr. Thomas Mack. He is professor in the Department of Preventative Medicine and Pathology at the University of California Keck School of Medicine.

To my right is Dr. --

CHAIRPERSON MACK: Southern California.

DEPUTY DIRECTOR ZEISE: What did I say? USC school. Sorry.

So to my right is Dr. David Eastmond, who is Professor and Chair of the Department of Cell Biology at the University of California at Riverside.

And then to his right is Dr. Joseph Landolph, who is an associate professor of molecular microbiology and immunology and pathology at the USC Keck School of

Medicine and associate professor of molecular pharmacology and pharmaceutical science at the USC School of Pharmacy.

To Dr. Mack's left is Dr. Shanaz Dairkee. She's senior scientist at the California Pacific Medical Center and a consulting professor for the Stanford University School of Medicine.

To her left is Dr. Jason Bush, an associate professor of cancer biology at the California State University Fresno.

To his left is Dr. Peggy Reynolds, who is a senior research scientist at the Cancer Prevention Institute of California and a consulting professor at the Stanford University School of Medicine.

And to her left is Dr. Luoping Zhang. She is an associate adjunct professor of toxicology at the School of Public Health at the U.C. University of California at Berkeley.

So welcome, everyone.

Now I'd like to introduce staff.

So there's Dr. John Budroe, who recently left the cancer toxicology and epidemiology section as Chief to return to as Section Chief of the Air Toxicology and Risk Assessment Section.

Next to John is Dr. Patty Wong, who we're welcoming as the new Section Chief for the Cancer

Toxicology and Epidemiology Section. And that section is the one that generates documents and materials for the hazard identification deliberations of this Committee.

Next to Patty is Dr. Martha Sandy, who is the Branch Chief for the Reproductive and Cancer Hazard Assessment Branch.

Next to Martha is Carol Monahan-Cummings, our Chief Counsel.

Next to her is Feng Tsai -- Dr. Feng Tsai, Gwendolyn Osborne, Jennifer Hsieh, Karin Ricker, and Kate Li. And these are all members of the cancer toxicology and epidemiology section. So welcome, everyone.

For logistics, I'd like to announce the meeting is being webcast. And so if people could speak into the mikes and introduce themselves as they speak if you're from the audience.

And we're going take a brief moment -- Carol, would you like to say something?

CHIEF COUNSEL MONAHAN-CUMMINGS: Good morning. We have Gordon Burns, who is the Associate Secretary for CalEPA, who is going to administer the oath to Chairman Mack of our Committee. And it will just take a couple minutes. But Dr. Mack has been reappointed to the Committee and we need to give him the oath before we proceed.

CalEPA UNDERSECRETARY BURNS: Should we do it right here?

I will read it to you and repeat. We'll try to get through this better than Obama and the Chief Justice. Raise your right hand.

I, state your name.

CHAIRPERSON MACK: I, Thomas Mack.

CalEPA UNDERSECRETARY BURNS: Do solemnly swear.

CHAIRPERSON MACK: Do solemnly swear.

CalEPA UNDERSECRETARY BURNS: That I will support and defend the Constitution of the United States.

CHAIRPERSON MACK: That I will support and defend the Constitution of the United States.

CalEPA UNDERSECRETARY BURNS: And the Constitution of the state of California.

CHAIRPERSON MACK: And the Constitution of the state of California.

CalEPA UNDERSECRETARY BURNS: Against all enemies, foreign and domestic.

CHAIRPERSON MACK: Against all enemies, foreign and domestic.

CalEPA UNDERSECRETARY BURNS: That I will bear true faith and allegiance to the Constitution of the United States.

CHAIRPERSON MACK: That I will bear true faith --

I missed the last word.

CalEPA UNDERSECRETARY BURNS: And allegiance.

CHAIRPERSON MACK: And allegiance.

CalEPA UNDERSECRETARY BURNS: To the Constitution of the United States.

CHAIRPERSON MACK: To the United States.

CalEPA UNDERSECRETARY BURNS: And the Constitution of the State of California.

CHAIRPERSON MACK: And the Constitution of the State of California.

CalEPA UNDERSECRETARY BURNS: That I take this obligation freely.

CHAIRPERSON MACK: That I take this obligation freely.

CalEPA UNDERSECRETARY BURNS: Without any mental reservation.

CHAIRPERSON MACK: Without any mental reservation.

CalEPA UNDERSECRETARY BURNS: Or purpose of evasion.

CHAIRPERSON MACK: Or purpose of evasion.

CalEPA UNDERSECRETARY BURNS: I will well and faithfully discharge the duties on which I'm about to enter.

CHAIRPERSON MACK: That I will faithfully

discharge the duties on which --

CalEPA UNDERSECRETARY BURNS: I'm about to enter.

CHAIRPERSON MACK: To enter.

CalEPA UNDERSECRETARY BURNS: Thank you.

CHIEF COUNSEL MONAHAN-CUMMINGS: Thank you, Gordon.

CalEPA UNDERSECRETARY BURNS: Thank you very much. Congratulations.

CHIEF COUNSEL MONAHAN-CUMMINGS: Sorry for the interruption.

DEPUTY DIRECTOR ZEISE: We'll resume with a few logistics before I turn the meeting over to Carol for some introductory remarks.

In terms of logistics, drinking fountains and rest rooms out the back door and to the left. Emergency exits are clearly marked at the door here, at the back door, and the side door. And there is a cafeteria downstairs.

Okay. So now, Carol, would you like to make some introductory remarks?

CHIEF COUNSEL MONAHAN-CUMMINGS: Sure. I wanted to point out -- and you may have noticed some of the logistics are a little bumpy today. But the primary reason for that is that we had two really very long-term staff with the Implementation Unit Prop. 65 that retired

in the summer. One was Cindy Oshita, who had been with the office for probably over 30 years, and the other one was Susan Luong who had been with the office over 20 years I believe. So they had supported this Committee and all the background stuff for so many years that we all took them somewhat for granted.

And so the supervisor for that group has been working on filling those two positions. And currently, they are not filled, but we're hopeful that they will be the next time this Committee meets. Right now, we're using backup folks. And I wanted to introduce Monet Vela, who is over here at the computer. She's done really hard work trying to cover the positions of three people. And so you may have gotten some e-mails from her. Other staff, my staff counsel Fran Kammerer. We've got -- I don't know if Barbara Moseman -- Barbara is not here. She's our legal assistant has helped a lot. A number of other OEHHA staff have pitched in. So I wanted to, even though they're not here, thank Cindy and Susan and also to thank our staff for pitching in.

So I just want to make the usual comments I make at the beginning of the meeting. I wanted to remind the Committee that you have listing criteria that was adopted by the Committee and you have copies of that in your binders today. And you were sent that with the other

materials along with the information on the chemicals that we're discussing today.

Your listing decision should be based on that criteria and your own scientific expertise, and not considering the future impact of the listing. For example, whether or not a warning might be required for a chemical exposure sometime in the future or how a listing might impact a particular industry or business.

The clearly shown standard that you have for the listings under this Committee is a scientific judgment call. It's not a legal standard of proof. Sometimes folks want to make it sound like it's beyond a reasonable doubt, like in a criminal case, and that's not the case. It's essentially a weight of the evidence standard.

Also, the Committee can decide and often does to list a chemical based on only animal evidence of carcinogenicity. The chemical does not need to be shown to be a human carcinogen. And you don't need to consider whether current human exposures to the chemical are sufficiently high enough to cause cancer in humans. So what you're actually doing is just identifying chemicals that are known to cause cancer, whether in animals or humans. The only caveat to that obviously is if there is no possibility that the chemical could cause cancer in humans, even though it does in animals. Sometimes there's

some theories about mode of action, but I'll leave that up to you all to figure out. I'm not a scientist.

The members of the Committee are appointed by the Governor because of your scientific expertise and you don't need to be feel compelled to go outside that charge.

So today, you have the options of considering listing chemicals, chemical groups, or declining to list or you can defer that decision on listing or not listing to another meeting if you feel like you don't have enough information to make a decision today. So you're not required in any manner to make a decision today if you're not comfortable.

So any questions on that? Okay. Thank you.

CHAIRPERSON MACK: Well, I'll add my welcome given by Lauren. For those of you who are here, we'll get on with the issue.

We have two groups the dibenzanthracenes and a group of nitrosomethyl-n-alkylamines. And the one thing that's novel to some extent for the deliberation of the Committee is that we'll begin by thinking about them as a group. And if the Committee feels like that the evidence suggests they could be listed as a group, we will list them as a group. If they're not, we'll take them individually. And as Carol said, we have the option of not listing them at all or not deciding.

So with that, let's begin with the dibenzanthracenes. And the first -- first of all, I'll turn to Dr. Landolph and Dr. Dairkee will discuss those when the time comes. And Dr. Bush and Dr. Zhang for the nitrosamines.

So Martha, tell us what to do next. Or actually do it.

DR. SANDY: Thank you, Dr. Mack.

I wanted to say a few things for the members that weren't on the CIC back in 2011, just so they have some background on where these chemicals came from.

So back in 2011, we brought to the CIC the chemical group dibenzanthracenes and the two chemical isomers in that group that are not already listed under Proposition 65 for ranking by your Committee. And at that time, the CIC ranked both the group and the individual isomers not already listed as having a high priority for selection and hazard identification document preparation.

So in 2011, shortly after your meeting, OEHHA -- we announced that we had selected the dibenzanthracenes and those two isomers not already listed for hazard identification document preparation. And we also issued a request for relevant information on the assessment of the evidence of carcinogenicity of these compounds. And no information was received at that time. So that's the

background on why we're bringing these two today.

And now I'll turn it over to Dr. Wong, who will introduce her staff.

DR. WONG: Good morning, Dr. Zeise and CIC members.

I would like to introduce the presenter today for in order of presentation, Dr. Feng Tsai, Dr. Gwendolyn Osborne, and Dr. Jennifer Hsieh. They will present evidence on the chemical dibenzanthracenes.

(Whereupon the following overhead presentation was given.)

DR. TSAI: Good morning. My name is Feng Tsai. Today, we are here to present the evidence on the carcinogenicity of dibenzanthracenes (DBAs). This presentation is an abbreviated version of the data that were reviewed in the hazard identification materials. These materials were prepared to assist the CIC's consideration of listing the DBAs as a group or listing individual chemicals within the group that are not already on the Proposition 65 list.

--o0o--

DR. TSAI: Here's the overview of today's presentation. First, we'll introduce the chemicals. Next we'll present the available carcinogenicity data, including animal bioassays, initiation promotion studies,

and other relevant data, such as genotoxicity, metabolism, and structure activity comparisons. We'll also present information on possible carcinogenic mechanisms and end the presentation with a brief summary of evidence.

--o0o--

DR. TSAI: DBAs are 5-rings PAHs with a common anthracene core. There are three isomers in this group. Each isomer has two additional benzene rings attached at different carbon bonds of anthracene. Here's the chemical structure of anthracene with the naming scheme.

The first DBA isomer is dibenz(ah)anthracene. The next isomer is dibenz(ac)anthracene. And the third one is dibenz(aj)anthracene.

These isomers share similar chemical properties. For example, they are lipophilic with low water solubility.

In addition, each isomer contains at least two or more "bay region" structures that are important for the formation of reactive metabolites, such as diol epoxides. Bay region theories have been proposed to predict the carcinogenic potency of PAHs.

Throughout our presentation, we will use the short-hand terms "ah" isomer, "aj" isomer, and "ac" isomers to refer to the different chemicals within this group.

--o0o--

DR. TSAI: This slide presents the cancer classifications reviews from other agencies. Usually, we present this information at the end of the talk. But because one isomer in this group, the ah isomer, is already listed under Proposition 65, we'd like to bring this information up now.

The ah isomer, with its extensive data, is also classified as a carcinogen by NTP, IARC, and USEPA. In fact, the ah isomer was the first pure chemical shown to be carcinogenic in animal studies as early as 1930.

The ac and aj isomers are classified by IARC as Group 3 chemicals.

None of these agencies reviewed DBAs as a group.

Since the ah isomer is already listed, our presentation will focus more on the evidence available for the ac and aj isomers. The ah isomer data will be presented briefly with the ah isomer colored in brown to show in the slides that this is a listed carcinogen.

--o0o--

DR. TSAI: DBA are products of incomplete combustion or pyrolysis. Emission sources are listed here, such as from cooking or smoking. Human exposure can come from contaminated air, food or water.

There are no commercial uses of DBAs. They are

used only for research purposes.

From biomonitoring studies, DBAs have been found in human tissues and also in wildlife. This slide shows the carcinoginicty data in human and animal studies.

--o0o--

DR. TSAI: No human data were identified for the pure DBAs, however, there are many epidemiology studies demonstrating that PAH mixtures containing DBAs, such as coke-oven emissions, are carcinogenic.

For animal data, ah is the most studied isomer. It has been shown to induce tumors at multiple sites in multiple species by multiple routes. Positive tumor findings for the ah isomer are summarized in Table 4 of the HID.

In contrast, the ac and aj isomers have limited animal data, only tested in mice. The ac isomer has a total of 9 animal bioassays conducted by three different routes-- dermal, subcutaneous and intraperitoneal injection.

The (aj) isomer only has two mouse bioassays, one by the dermal route and one by the subcutaneous route.

--o0o--

DR. TSAI: First, we'll present three ac bioassays by the dermal route. The first two studies done in '62 and '68 did not show treatment-related tumors,

possibly due to less than lifetime dosing and study period, and small numbers of animals. For example, Finzi et al, study was conducted in 20 animals and observed for 25 weeks.

--o0o--

DR. TSAI: The third dermal study applied the ac isomer in Swiss mice twice a week for 65 weeks and observed the animals for life. The controls of 20 animals were treated with solvent for 100 weeks. Skin tumors were observed only in the ac treated group. The first tumor was observed at 60 week, suggesting the previous two dermal study with a study duration of 25 or 56 weeks may not have been sufficient to permit the observation of treatment-related tumors.

As shown in this table, there were statistically significant increases of skin squamous cell carcinoma and combined carcinoma and papilloma in the treated mice, compared with no skin tumor in the controls.

Based on our pathology reviews, skin tumors are considered rare in mice, usually with background incidence less than 1%. Moreover, Lijinsky, et al, reported that historical control of Swiss mice were untreated and solvent treated only control Swiss mice was really rare. Moreover, the authors state that the control mice rarely develop an occasional skin papilloma but never a

carcinoma.

--o0o--

DR. TSAI: Next, we'll present five subcutaneous bioassays for the ac isomer. The first two bioassays did not report any treatment-related tumors, possibly due to limitations in study design.

In the third study, Kouri, et al, administered a single injection of the ac isomer, tested at two dose levels to three strains of mice with different binding affinities for aryl hydrocarbon receptor (AhR). Two strains have high affinity for the AhR and the third strain has low affinity for the AhR. At 12 months, one rare skin fibrosarcoma was observed at high dose groups in each of the high-affinity strains. The study did not report tumor incidences in the control.

--o0o--

DR. TSAI: The third route of exposure was done by ip injection for the ac isomer. Two control groups were used in this study, one vehicle control group and one positive control group. The ac isomer was administered during the first two weeks of life to male mice. As shown in the table, the ac treated animals have a statistically significant increase of liver adenomas observed at 12 months. Liver adenomas may progress to liver carcinomas.

--o0o--

DR. TSAI: Now we'll present bioassays data for the aj isomer. There are only two bioassays identified, one by dermal application and the other by subcutaneous injection. Both are conducted with female Swiss mice.

In the dermal application study, the aj isomer was applied twice a week for 60 to 81 weeks at two doses and observed for life in group of 30 mice. The control group had 20 animals to begin with, and 14 animals survived to week 60. Survival in the low dose group was statistically significant lower than that of the control, while survival in the high dose group was similar to that of the control. No explanation was given in the paper.

As shown in the table, the aj-treated groups show increases in skin papilloma and squamous cell carcinoma. These increases were statistically significant by pairwise comparison for carcinoma in the high dose and for combined papilloma/carcinoma in both high- and low-dose groups. In addition, statistically significant dose response relationships were observed for both carcinoma and combined papilloma/carcinoma by the exact trend test.

In summary, this dermal bioassay shows treatment-related skin tumor increased both by the pairwise and by trend test.

The second study administered the aj isomer by a single subcutaneous injection to 25 female mice and

observed for life. Though not statistically significant, rare skin sarcomas were observed in 3 of 15 ac treated animals, compared with none in the solvent control group. The author did not specify whether or not these are injection-site sarcomas.

--o0o--

DR. TSAI: Next we'll present data from the initiation promotion studies with ac and aj isomers and their metabolites.

First, this slide summarized the results for the ac isomer and some of its metabolites. The first column lists key study design elements, such as the mouse strain tested and the study duration. The ac isomer and its metabolites were studied in the 2-stage model using different strains of mice. All studies used TPA as tumor promoter, except the first one, which used croton resin.

Results noted with a positive sign indicates statistically significant initiating effects observed in the ac or ac metabolite-initiated group, compared with promoter-only group. A "+/-" sign indicates that some tumor initiating activity was observed, but either the increase in tumor incidence did not reach statistically significance at p=0.05, or there were no control data available for statistical comparison.

For example, the fifth study listed in the table

by Scribner and Scribner 1980 shows 75% of ac-initiated mice developed skin papilloma, but there were no control data available for that study. As discussed in some detail in the HID and summarized in this table, the majority of the studies on the ac isomer show evidence of the tumor-initiating activity.

In addition, two of the ac metabolites tested were also skin tumor initiators, with a third metabolite showing some initiating activity, although the increase in tumor incidence did not reach statistical significance.

--o0o--

DR. TSAI: We won't report all studies. Here we show an example of an ac isomer initiating promotion study.

The ac isomer was applied as a tumor initiator, followed by 56 to 58 weeks of TPA promotion. As shown in the table, the ac/TPA treated group had a statistically significant increase in papilloma, compared with the TPA only group. The ac/TPA treated group also had increased skin carcinoma incidence, but the increase did not reach statistical significance.

--o0o--

DR. TSAI: Next we'll present the results on the aj initiation promotion studies. All studies listed here used SENCAR mice, which is considered the most sensitive

strain for the initiation-promotion model. All results show statistically significant tumor initiating activities for the aj isomer and its two diol or diol epoxide metabolites.

--o0o--

DR. TSAI: Here's an example of the ac initiation promotion study.

Harvey, et al, tested the aj isomer and two of its metabolites as initiators, followed by 14 weeks TPA promotion. All three chemicals tested show initiating activity, with increased numbers of papillomas per mice and a statistically significant increase of papilloma incidence, compared to the vehicle-initiated group.

In addition, the 3,4-diol 1,2-epoxide metabolite showed greater initiating activity than the parent compound on an equimolar basis.

--o0o--

DR. TSAI: Next let's look at other relevant data on DBAs.

The next two slides present a brief summary of the genotoxicity data. A more complete review is in the HID.

The Ah isomer, a listed carcinogen, is genotoxic, as shown in a number of different assay systems listed here.

The ac isomer induces bacterial DNA damage, and both the ac and aj isomer are mutagenic in bacteria assays and have tested positive in multiple in vitro and in vivo assays that will be shown in the next slide.

In addition, metabolites of each of the three DBA isomers are also genotoxic. Some metabolites are more potent than the parent compounds.

--o0o--

DR. TSAI: This table summarizes the gentoxicity results for the ac and aj isomers and their diol and diol epoxide metabolites.

The first column lists the different genotoxicity assays, and the results are presented for each of the isomers and their metabolites.

First, let's look at the parent compounds. Both the ac and aj isomers induce bacterial gene mutation or DNA damage. Both form DNA adducts in vitro and in vivo. And both induce mutations in mammalian cells, including oncogene mutations in mice. In addition, the ac isomer also induces UDS, tested positive in mouse micronucleus assay and induces somatic mutations in fruit flies. The aj isomer has not been tested in these assays.

Next, let's look at the metabolites. Metabolites of both isomers are tested positive in bacterial assays and form DNA adducts in vitro and in vivo. In addition,

ac metabolites induce sister chromatid exchange; aj metabolites induce oncogene mutations in mice.

In summary, this table shows that both the ac and aj isomers and their metabolites are genotoxic in multiple short-term tests.

--o0o--

DR. TSAI: Studies on the induction of morphologic changes by the DBAs are presented in this slide.

First, the ah and ac isomers were tested positive in vitro cell transformation studies. In general, there is good correlation between the results of in vitro cell transformation studies and in vivo carcinogenesis in rodents. The aj isomer was not tested.

In addition, one in vivo study conducted in rats reported that the ac isomer induced preneoplastic morphological changes, such as epithelial hyperplasia and squamous metaplasia in transplanted rat tracheas, exposed by pellets containing ac isomer.

--o0o--

DR. TSAI: The author stated that ac isomer caused severe and long-lasting epithelial and submucosal change. Next let's look at the pharmicokenetics and metabolism. The detail is in the hazard identification materials. Here are some highlights.

ADME evidence comes from many in vivo and in vitro studies, mostly of the ah isomer.

DBAs are absorbed slowly by dermal application and subcutaneous injection. Absorption is faster, within hours, by gavage.

Once absorbed, DBAs are rapidly distributed within the body. Major compartments are the gastrointestinal tract or liver, depending on the administration route.

Multiple metabolic pathways and metabolites were identified. Different enzymes, such as epoxide hydrolase and cytochrome P450s are involved in the metabolism of the DBAs.

Similar metabolites, such as diols and diol epoxides, were identified for each of the three isomers.

DBAs are mainly excreted in the feces and urine within days.

--o0o--

DR. TSAI: This slide shows some of the DBAs' metabolites. For the ah isomer alone, more than 30 metabolites have been identified, including quinones, phenols, and diol epoxides.

This is a 1,2-diol metabolite with two hydroxyl groups. Diols are common metabolites of all three DBA isomers as shown in the red circle here.

This is an diol epoxide metabolite from ac isomers. Similar diol epoxide metabolites were also found for the other two isomers. Diol epoxides can be further metabolized by epoxide hydrolase to form bis-diol metabolites. This is the bis diols metabolites for the aj isomers. Similar metabolites was identified for the ah isomer.

--o0o--

DR. TSAI: This slide represents some metabolic pathways for the ah isomer, showing enzyme-mediated formation of diols, diol epoxides, and bis diols. These metabolites were all identified in either in vivo or in vitro assays, except for those two marked with an asterisk.

First, the ah isomer is metabolized by P450 to an epoxide, then with epoxide hydrolase to form a diol, and further metabolized to different diol-epoxides or bis-diol epoxides.

Reactive carbonium ions are one possible end product. These and many other DBA metabolites, such as the diol epoxides, are all genotoxic.

The metabolic pathway for the ac and aj isomers are not as well understood. But all three DBAs share similar metabolites, including the diols and diol epoxides, which indicate that similar metabolic pathways

are likely to occur.

Next Dr. Osborne will present on structure activity comparison with related compounds.

--o0o--

DR. OSBORNE: We chose 6 structurally-related non-substituted PAHs to compare to the DBA isomers based on the following criteria:

They needed to contain four to six aromatic rings, with at least three in a linear configuration, at least 1 bay-region structure, and were tested in animals.

We found that almost all are genotoxic and carcinogenic, form genotoxic and carcinogenic metabolites, are on the Proposition 65 list of carcinogens and are classified as carcinogens by IARC as either Group 1, 2A, or 2B except for Dibenzo[a,e]Pyrene.

--o0o--

DR. OSBORNE: Here are the related PAHs. On the left, we have the three DBA isomers, each of which has five rings. The top middle we have benzo[a]pyrene, also with five rings. Then in the middle is benz[a]anthracene with four rings. Then there are four dibenzopyrene isomers, each of which has six rings.

--o0o--

DR. OSBORNE: This table compares the PAHs. The first three rows are the DBA isomers. Below that are the

six related compounds: Benzo[a]pyrene, benz[a]anthracene, and the four dibenzopyrene isomers.

As you've already heard for the DBA isomers, each compound is genotoxic. Each is also a skin tumor initiator in initiation-promotion studies.

All the comparison compounds, except dibenzo[a,e]pyrene, also form diol or diol epoxide metabolites that are genotoxic and skin tumor initiators.

Additionally, tumors have been observed in several sites in mice following exposure to these compounds. The most common sites are the skin, liver, and lung. Liver tumors have been observed for all but the a,j isomer and dibenzo[a,e]pyrene. Lung tumors have been observed in mice for all but the ac and aj isomers and dibenzo[a,e]pyrene. Some of these compounds have also induced lung tumors in other species, such as rats and hamsters, as indicated by the footnotes.

Overall, you can see that there are numerous similarities in biological activity between the DBAs and other PAHs.

--o0o--

DR. OSBORNE: As additional evidence for the carcinogenicity of the ac and aj isomers, we applied Quantitative Structure Activity Relationship models, also known as (QSAR) to predict carcinogenicity.

In general, QSAR models correlate physical and chemical properties of related compounds to their biological activity to predict the toxicity of chemicals for which data are lacking.

In order to choose from the many different models that have been developed, we used published sets of guidelines to select four publicly available models. These were VEGA, which is a platform containing the CAESAR and ToxTree models, Lazar and QSAR Toolbox.

We also used 2 additional models published in the scientific literature by Barone, et al, and Vijayalakshmi and Suresh. These papers correlated electronic properties of PAHs with carcinogenicity. We did not actually run these two models. The results for the DBA isomers and the other PAHs were published in these two papers. We did run the models VEGA, Lazar, and Toolbox, and the results are presented in this table.

--o0o--

DR. OSBORNE: Overall, all models predicted both the ac and aj isomers to be carcinogenic.

The exception was Barone, et al. The aj isomer did not meet the criteria for strong or moderate carcinogenicity, nor did it meet the criteria for inactive or weak carcinogenicity, so the prediction given in the paper was not clear.

However, the rest of the predictions were all made with good reliability according to various model parameters.

In conclusion, additional evidence for the carcinogenicity of the ac and aj isomers is provided by these QSAR model predictions.

Now Dr. Hsieh will present evidence on carcinogenic mechanisms.

--o0o--

DR. HSIEH: Thank you.

Move onto the carcinogenic mechanisms of dibenzanthracenes. The IARC monograph volume 92, published in 2010, discusses in some detail the available mechanistic evidence for individual PAHs, including each of the DBA isomers. The relevant pages of the monograph are included in the hazard identification materials as Attachment II.

The proposed mechanisms are genotoxicity, receptor activation, immune suppression, and alterations in regulation of cell growth. Additional mechanistic information that has become available since the IARC review, including data on the ah isomer from toxicogenomic studies and from the US EPA ToxCast testing program, is also summarized in the hazard identification document.

In today's presentation, we will focus primarily

on the two most well-studied mechanisms for the DBAs, namely, genotoxicity and Ah receptor-mediated mechanisms, as indicated in bold here.

--o0o--

DR. HSIEH: Next, we will look at genotoxic mechanisms. The genotoxicity of DBAs is dependent upon metabolic activation to form DNA reactive species. These reactive metabolites may form DNA adducts, or otherwise damage DNA, resulting in mutations and other genetic changes that lead to tumor formation. Several key types of DBA reactive metabolites are shown here:

First, carbonium ions can be generated from diol epoxides. For example, all three DBA isomers can form carbonium ions from their bay region diol epoxide metabolites. The strong carcinogenicity of PAH bay region diol epoxide metabolites has been recognized since the 70s.

Next, radicals are produced from one-electron oxidation reactions catalyzed by peroxidases or CYP450s.

Lastly, o- and p-quinone metabolites may bind directly to DNA, or undergo redox-cycling to generate reactive oxygen species, which in turn may lead to oxidative DNA damage.

--o0o--

DR. HSIEH: We'll now continue with our summary

of the evidence supporting a role for genotoxicity as a carcinogenic mechanism for the DBAs. This table summarizes the data on genotoxicity, mouse skin tumor initiating activity, and animal bioassay findings for each of the DBA isomers and for several of their diol or diol epoxide metabolites.

First, all 3 DBA isomers and the diol or diol epoxide metabolites shown here are genotoxic.

In addition to being genotoxic, all three DBAs are also skin tumor initiators and there are positive tumor findings in animal bioassays.

Two of the Ah isomer's diol or diol epoxide metabolites are also skin tumor initiators and have positive tumor findings in animal bioassays.

Three of the ac isomer's diol metabolites and two of the aj isomer's diol or diol epoxide metabolites are also skin tumor initiators. But, they haven't been tested in animal cancer bioassays.

--o0o--

DR. HSIEH: This slide highlights several lines of evidence suggesting that Ah receptor-mediated mechanisms are involved in the carcinogenicity of PAHs, including the DBAs. The evidence that DBAs induce Ah receptor mediated effects includes:

Several studies of cytochrome P450 enzyme

induction associated with AhR. The particular CYP isozymes that are induced by DBAs are also capable of metabolizing DBAs to form genotoxic species.

Enhanced DNA adduct formation by the ah isomer has been observed in mouse skin 24 hours after dermal application in wild type mice, as compared to AhR knockout mice.

Enhanced skin tumor induction, by the ah and the ac isomers has been observed, as well as enhanced CYP1A1 induction, in mice expressing a high-affinity AhR, as compared with mice expressing a low-affinity AhR.

As discussed in the portion of the IARC 2010 monograph included as Attachment 2 to the hazard identification materials, a number of additional AhR-mediated signaling pathways are thought to be involved in PAH-induced carcinogenesis. The effect of the DBAs on these other pathways has not been studied, but studies of other PAHs have been conducted.

Briefly, AhR receptor activation by other PAHs has been shown to result in alteration of tumor suppressor genes and activation of some oncogenes, such as c-Myc, as well as cross-talk with other nuclear receptors, such as the estrogen receptor, and activation of p53-dependent or p53-independent pathways that suppress immune functions.

--o0o--

DR. HSIEH: This table summarizes findings associated with several of the possible carcinogenic mechanisms for the DBAs and for the most well-studied PAH, Benzo(a)Pyrene.

As discussed previously, there is strong evidence that all three DBA isomers and Benzo(a)Pyrene are genotoxic.

And that they can activate the Ah receptor, and that AhR-mediated effects are involved in skin tumor initiation and carcinogenicity.

All three DBA isomers and Benzo(a)Pyrene can alter cell growth. However, the data are limited for the ac and aj isomers reported in only one study for the ac, and aj isomers, in which a dose-dependent increase in cell proliferation was observed in rat liver epithelial cells in vitro.

--o0o--

DR. HSIEH: Lastly, immune suppression was found to be induced by the ac, and ah isomers and Benzo(a)Pyrene. However, the evidence for the ac isomer is limited to one in vitro study conducted on human T-cells. Currently, there are no data for the aj isomer on immuno-suppression.

Overall, the evidence suggests that all three DBA isomers are likely to act through similar mechanisms as

those proposed for Benzo(a)Pyrene, to induce tumors.

--o0o--

DR. HSIEH: In conclusion, this slide summarizes the tumor findings from animal studies of the three DBA isomers.

For the ac isomer, female Swiss mice exposed by dermal application were observed to have statistically significant increases in skin squamous cell carcinoma, and combined papilloma and carcinoma, as compared to controls;

In another study, a statistically significant increase in liver adenoma was observed at 12 months in male B6C3F1 mice, following neonatal i.p. injections.

In addition, the ac isomer and three of its diol metabolites are skin tumor initiators.

For the aj isomer, female Swiss mice exposed by dermal application were observed to have statistically significant increases in skin squamous cell carcinoma, and combined papilloma and carcinoma, with a dose-dependent trend, as compared to controls.

In another study, the induction of rare skin sarcomas were observed by subcutaneous injection in female Swiss mice.

In addition, the aj isomer and two of its diol and diol epoxide metabolites are skin tumor initiators.

And, as reviewed previously, the ah isomer, which

is already listed under Proposition 65, has been shown to induce tumors in multiple species, by multiple routes.

--o0o--

DR. HSIEH: To continue with our summary of the other relevant data:

All three DBA isomers and their metabolites are genotoxic:

They all tested positive in bacteria, and in in vivo and in vitro genotoxicity assays. And they all form DNA adducts.

Ah isomer and ac isomer induce cell transformation in vitro.

Ac isomer induces preneoplastic morphologic change in vivo in subcutaneous transplanted rat tracheas.

All three DBA isomers activate AhR-mediated pathways.

All three DBA isomers share strong structure-activity similarities with six comparison PAH carcinogens.

Lastly, the carcinogenicity of ac and aj isomers is supported by several QSAR model predictions.

This concludes our presentation on the carcinogenic evidence of the dibenzanthracenes. The evidence summarized here supports the CIC's deliberation on the listing of dibenzanthracenes as a group, or the

individual listing of Dibenz(ac)anthracene or Dibenz(aj)anthracene.

Thank you for your attention.

CHAIRPERSON MACK: Thank you.

Now we'll see if anybody on the Committee has any questions for the staff.

I have one question/observation. It looks as though the metabolites are pretty nasty, just like the DBAs are. And it also looks like that they're probably really widely distributed in the environment. They're excreted in feces, which means they must be very widely distributed even in commercial areas of California, for example. What we don't know, they haven't been tested as much in detail as the DBAs.

DR. TSAI: Yes, the environmental data they were available in the air, in the water. I think we present that in the chemical identity part. So they are identified in the occupational setting, in the cooking indoor environment, and also in the drinking water, in fresh water, in lake sediment, everywhere.

CHAIRPERSON MACK: They refer to lakes because somebody decided that would be an interesting thing to measure.

DR. TSAI: Yes or no. I think they are trying to characterize the contamination, because with their

long-lasting biodegradability issue.

CHAIRPERSON MACK: Anybody on the Committee have any questions for the staff?

Dr. Dairkee.

COMMITTEE MEMBER DAIRKEE: Even though there is such wide distribution of these things, there is no epidemiological data. Is it because everybody has such high levels of these compounds that who do you -- who is the control and who's the case and how do you do epidemiological studies in that occasion?

DR. TSAI: Dr. Reynolds may be a better person to answer the question. But from my basic understanding is that first DBA ah isomer was identified as carcinogenic in animals. So you couldn't possibly have pure chemical administer in human. And the difficulty of conducting epi data using the -- to relay the single chemical is that there's no -- you couldn't possibly -- because PAH mixtures are so hard to characterize, unless you have very high concentration like benzo(a)pyrene, for example. But even with benzene you have many co-exposure or co-contaminants. So it's hard to tease out the individual association. But Dr. Reynolds would provide better answer.

COMMITTEE MEMBER REYNOLDS: I think that's a very good answer. I think from the human health point of view

it's the complexity of these mixtures which makes it really hard to disengage in the human health study.

I actually have a question.

CHAIRPERSON MACK: I would think it's also very difficult to identify even exposure to the mixtures and the degree, because it's so universally spread that it would be hard to single out, as you said, distinguish between cases and controls because everybody is exposed to some extent. Only in the case of something like people who work in the -- the one that's mentioned here, people who work in the steel industry who get very, very heavy exposure to soot or to products of incomplete combustion. But then it's the whole mess that's going on.

Well, let's proceed. Joe, you're the first.

Sorry, Peggy.

COMMITTEE MEMBER REYNOLDS: I actually had a question, but I don't know maybe the discussants are going to address this. It wasn't quite clear to me the time, the trajectory in terms of time for how much of this evidence is new -- since a lot of the studies you cited are actually quite old, how much of this is new evidence since the last time this has been reviewed by any of these informative bodies? Do you know have information on that or sort of a general sense? Or is that something that you guys are going to discuss already? So is that a premature

question?

DR. TSAI: I don't have specific answer on how many new studies since the IARC 2010 review. But most all of the bioassay study and initiation promotion studies are very old. Done prior to 1990. And there are some new study on the Toxcast or some other relevant information on the DBAs. They are newer, like the newest study we found was 2013, but --

COMMITTEE MEMBER REYNOLDS: It's mostly old.

DR. TSAI: The majority of the bioassays and initiation promotion studies were very old.

CHAIRPERSON MACK: Yes. Dr. Zhang.

COMMITTEE MEMBER ZHANG: I have two small questions for confirmation.

On slide number eleven, Dr. Tsai, when they have the dose response as slide eleven, low dose and high dose, I heard you saying P strain, significant P strain. We don't have P strain study here. I wonder if that in the controls -- I just want to make sure my understanding is correct. Is that the stars on the control that means P strain?

DR. TSAI: The stars in the control groups shows the trend test P. One star meaning the P less than .05. And two star meaning that's less than .01.

COMMITTEE MEMBER ZHANG: That is correct.

Another question. Slide number 17, we are looking at the parent compounds and the compared with metabolites. For example, the first genotoxicity bacteria gene notation. My question is for parent compounds, do they add S9 to really indicate as a parent compound or in the testing system they already add S9? So that's basically my question. How do we know that the testing for parent compounds is truly correct?

DR. TSAI: So most of the bacteria gene mutation assays, they were done with S9. But there are some studies shown that they have -- they were conducted both with and without S9.

DR. SANDY: On Table 25 in the HID, you're talking about the bacterial data. Those tests for the AC isomer. And there are no positive tests in the absence of S9 for the AC.

So metabolic activation is needed and I could point you to the other table for the aj. What we're saying is the administrations of the parent compound, we're getting a positive result. And then as Dr. Tsai said, there were 30 metabolites have been identified for the ah isomer. I don't know how many have been identified for ac. There are a whole bunch of metabolites. They only tested a handful. You know, we don't know which metabolites are key. Perhaps there are multiple

metabolites, all active.

CHAIRPERSON MACK: David.

COMMITTEE MEMBER EASTMOND: Couple questions for you.

One has to do with the data on slide number ten, which is the ac isomer tested by i.p. injection. Now, what you've done is a statistical comparison to between the adenoma frequency and vehicle controls and essentially the ac isomer treated animals.

Did you look at the statistical significance when you combined the adenomas and carcinomas together? Do you typically look at these individually or do you usually combine them?

DR. TSAI: We will combine them if we are sure that it's the simple summation. Because sometimes one animal could have both adenoma and carcinoma. In the paper, the original paper did not report a total number of adenoma and carcinoma. And we don't have any supporting evidence. We wouldn't do our own summation.

COMMITTEE MEMBER EASTMOND: I had another question. This is on table number eleven, which I consider one of the -- probably one of the more important pieces of evidence. I just found -- I actually went back to the original paper on this. I found something surprising. The author did not consider the low dose to

be significant the increase and the high dose considered to be something like borderline significance. Do you have any reason why? Did you have any understanding as to why there were sort of different call than you've seen a very strong response versus what the authors themselves said.

DR. TSAI: If I remember correctly in the original paper, they didn't report the statistical test. We did our own comparison, and the result are based on the P value we have either by the trend or by the comparison.

DR. SANDY: If I can interject just for clarification. You're talking about Table 11 in the document or slide 11?

COMMITTEE MEMBER EASTMOND: Slide 11 which corresponds to Table 10, I believe. Slide 11.

DR. SANDY: For the dermal application or the injection?

COMMITTEE MEMBER EASTMOND: It's the dermal application.

DR. SANDY: Okay. Thank you.

DR. TSAI: In the original paper, they only report tumor number without statistical testing. And we extracted the number from the paper and compiled the table and then conduct our own pairwise comparison. The statistical significance are based on the P value equal to .05. So clearly in the high dose group, the tumor

response is higher. Maybe that's why.

COMMITTEE MEMBER EASTMOND: Well, I mean, just as you know, that study is plagued by very high control mortality and controls and the treatment. So I mean, I actually think you did as good as a job as can you do, given the data you're working with. And I agree with the conclusions. But I found it surprising when I looked through the discussion the authors described that was their description of the results, was even the one that looks very, very strongly increased, they consider to be borderline. And it may be in relationship to they were comparing the ah isomer, which was much more potent or something. But that was the description.

COMMITTEE MEMBER LANDOLPH: I have one question. Why do you think that the EPA and IARC and NTP did not bite on these compounds? Why they call them non-classifiable today? Do you have a feeling?

DR. SANDY: If I can jump in. The only agency that's looked at this is IARC has looked at the two isomers. EPA and the others have not looked at them.

COMMITTEE MEMBER LANDOLPH: And then one more question. This is such a huge amount of material. I read through it a number of times. I didn't get a chance to go onto web and look at your nice disc. Did you find a lot of dose responsive data for the tumorgenicity of these two

isomers? I didn't see a lot of dose response data here.

DR. TSAI: We present all the evidence we could find.

COMMITTEE MEMBER LANDOLPH: The answer is no.

DR. TSAI: I would say with the limited bioassays, this one does show good dose response relationship, even with their high mortality rate in the low dose.

COMMITTEE MEMBER LANDOLPH: Not good dose response data by my standards. There was not a lot of doses tested. It's not your fault. It's just a fault of data. Okay.

CHAIRPERSON MACK: So no more questions, then let's go to Joe and give us your discussion.

COMMITTEE MEMBER LANDOLPH: I think staff did a fantastic job putting all data together. It is a plethora of data, a huge of amount of data.

Your Table 37 is a very nice table. There is pluses all the way down the line for genotoxicity for parent. There is genotoxicity for diol, other diol metabolites. The parents are compounds. The isomers are tumor initiators, which you would expect because they make diol or diol epoxide metabolites and these are mutagenic skin tumor initiators as well. This all seems to fit fairly well together for me.

Table 38, which is very nice summary of the table you made, also on page 60 shows very nicely that the ac compound causes skin and liver tumors in the males and that the aj compound causes skin tumors. And I think the database is not as extensive as that dibenz(a,h)anthracenes. Dibenz(a,h)anthracene was discovered in 1930 and has about 50 assays and as you point out haven't had this courtesy extended to them yet.

The history of these compounds very similar to benzopyrene in many ways which was discovered in 1932. And of course, we know so much about benzopyrene. You have K region epoxides. You get diol apoxides. And these compounds you also get bay region diol apoxides. You get K region epoxides and sometimes phenolic metabolites which are later metabolites again into K region epoxides.

So this data seems to fit together pretty well. I think the QSAR is pretty convincing and the aromaticity of these compounds drives everything. I'm pretty convinced that they're metabolized to K region epoxides and bay region biepoxides very complex manner. And they have combined with the DNA and the diol epoxide metabolites do. They're quite genotoxic across a spectrum of assays. While the database on carcinogenicity is not quite as extensive as dibenz h, there are positive assays there. So I think I'm convinced they're carcinogens. I

would expected them to be carcinogens. We are arguing over a database that's not as robust as dibenz(a,h)anthracenes, but may not be as robust as that. I think there's enough here for me to pull the trigger on it.

CHAIRPERSON MACK: Thank you, Joe.

Dr. Dairkee.

COMMITTEE MEMBER DAIRKEE: I agree with Dr. Landolph. The staff has developed an incredibly thorough and well-organized document, which I learned a lot from it. It was very, very well done. And in fact, I was inspired for some possible future research directions. So I must congratulate the staff on putting that together.

So it's very clear that the body of evidence for the carcinogenicity of the DBAs is quite longstanding and it's vast. Yet, only one of these are the most data is listed as a probable carcinogen by IARC. And as shown in Table 4, the ah tumor development occurs in whichever tissue it is injected into, demonstrating there is system-wide susceptibility for these chemicals. And it's not an association with the differentiation status of a particular tissue or cell type. And so most likely due to massive genetic damage, which means to me that the other isomers would have similar effects as well, although fewer injection sites have been tested.

So as someone who doesn't routinely work with experimental animals, it was quite curious to me that the researchers in this field of animal carcinogenicity will examine tumorgenicity of a group of chemicals with different solvents, and which I think results in a lot of data variability, so which is why I feel like some people who dissolve some studies where they dissolve -- where they use benzene to dissolve the DBAC and aj found no tumors. So the only group that it was very curious that the only group that consistently found tumors was using acetone as a solvent. So that's very interesting. And they saw -- Lijinsky saw the tumor development with the acetone solvent both subcu and dermal. Dermal or whatever they do.

And in fact, there was another study where they used TPA, which was dissolved in acetone as well. And that's another study where they found that tumors occurred. So it seems to me like acetone is very synergistic with these isomers. And maybe that's an explanation for the survival issue that we were talking about in the Lijinsky paper of 1970 where they found that the low dose group was significantly better than the control group, survival-wise. So they were finding in that paper that the high dose group had a similar survival to the control group. And I'm just thinking that possibly

it's the acetone toxicity related to survival which is alleviated by the high dose DBA, but not by the low dose. The toxicity of acetone is alleviated by the high dose DBA. But up to a certain point when the tumors develop, then, of course, they develop more frequently in the ah -- I mean aj in the high dose aj and ac isomers.

Then the pre-treatment with the -- in conjunction with other carcinogens which reduces the carcinogenic effects was very difficult to understand mechanistically why that would happen. It's really at this point it doesn't make any sense.

For in the in vitro genotoxicity data, I felt that the DBA concentrations used were very high. They were as high as one milligram or .2 millimolar. And I'm not sure if such levels of exposure occur environmentally. But even lower levels were shown to cause mutation induction. So it does happen. Genotoxicity does happen at lower levels in some of the studies. It's very clear.

And similarly in the cell transformation studies, I felt that -- and the tracheal transplant studies for the in vivo morphological changes, I felt they had used fairly high concentrations, around one mg per ml to achieve the positive results.

Overall and in terms of metabolism, both the isomers are metabolized and the metabolites are genotoxic.

The chemicals have a fairly long half life. So it's bad news.

In terms of comparison with PAHs, it was very helpful to see the similarities and that they are quite striking. The QSAR modeling is also predictive of carcinogenicity. And together with all the other hard end points shown experimentally, it's fairly convincing that the assays are common to all three isomers, show similar data. And just because the AH isomer has been studied more extensively, there are more data points available. Absorption assays were -- not data was not very clear on the two other isomers.

DR. TSAI: There is no data.

COMMITTEE MEMBER DAIRKEE: There is no data. The chemical hangs around for so long and the chemicals, both of them and ah, they cause so many pertubations, it is indeed a cause for concern. And based on the structure of ac and aj, there is really no reason to believe that their absorption and distribution would be any different than ah. In fact, the solubility profile suggest they might even distribute more extensively at lower concentrations because they seem to be more soluble in the ac and aj.

Anyways, I feel that because they're present everywhere and there is a great likelihood of over-exposure, even though the epidemiological data is not

available for any of the isomers, there is enough hard data from all the assays that these are toxic chemicals, both of them.

CHAIRPERSON MACK: Is there any comment from the Committee?

Joe.

COMMITTEE MEMBER LANDOLPH: The studies in which they put these in benzene are likely older studies. Nobody does that any more. Because benzene itself causes acute myelogenous leukemia and other types. That is a red-herring. It's most likely due to competitive substrate effect where benzene is being metabolized instead of the other compound. And the acetone itself is not toxic. It's a common solvent. It's not toxic at all. In fact, that's why it's used. It's not having any effect in these experiments.

COMMITTEE MEMBER DAIRKEE: I just wanted to comment that in the Table 9, there is a decline in the survival even in the control with acetone.

COMMITTEE MEMBER EASTMOND: But no one knows why.

COMMITTEE MEMBER DAIRKEE: Yeah. I agree.

CHAIRPERSON MACK: If there is no more -- David.

COMMITTEE MEMBER EASTMOND: Throwing my two cents.

Personally, I find the evidence -- the cancer

evidence by itself is pretty marginal but probably adequate. But when you put the cancer bioassay data together with all the other supporting information, genotoxicity, structure activity relationships to the initiation promotion, I find the evidence becomes certainly sufficient for me to list as a group, both of them. This is older data. It's not very good data. Prone to problems, survival problems. But in spite of that, there's still enough I think of a picture here kind of that Joe had mentioned I would certainly think that it should be listed.

CHAIRPERSON MACK: Yes. Dr. Bush.

COMMITTEE MEMBER BUSH: If David gets his two cents, I will throw in my two cents as well.

I, too, agree that the data is compelling with respect to the bioassays -- animal bioassays and supporting data from the genotoxic studies. What surprised me is that I think I remember a number of something like you sifted through 450 different citations in terms of your searches or something like that. You found 450 papers. And it astounds me that there is no human data out there in the epidemiological studies of any sort. Even if we do have a common problem in the population of a saturation of this class of chemicals, I think it's worth actually investigating what is the steady

state level that was present in human tissues.

CHAIRPERSON MACK: I think it's fair to say there is no human data. It's just the human data is based on multiplicity of compounds. I mean, smokers gets this stuff. People who work in coke ovens get this stuff. If you were to eat soot, you would get this stuff. And there aren't a lot of soot eaters to make a cohort out of. It's just tough.

COMMITTEE MEMBER BUSH: Right. But I think that begs the question: What is the presence in the general population of these chemicals?

CHAIRPERSON MACK: I think that is an interesting question. You did the best you could with the available data. We really don't have quantitative information on how much of this stuff is in the things we eat every day and the things we're exposed to. But that's not the job of this Committee. But it would interesting to know exactly how much of it is around.

DR. TSAI: Can I clarify? When we say there is no human data, we mean there is no human cancer epidemiologicaldata for the pure DBAs. There are human biomonitoring data from the blood, from the placenta, also from the food, marijuana, emission, cigarette smoke emissions. For example, in the paper or studies we reviewed, they report the DBA's concentration in food in

different Italian restaurant and Indian food. So we do have number from the -- for the current or within 20 years of DBA concentrations in different environmental mediums. We just don't have the human epi data with the pure DBAs.

DR. SANDY: I'll add that people are exposed not just to dibenzanthracenes, but other PAHs are all formed in the same processes. And that's another difficulty in constructing an epidemiological study to look only at the DBAs when they occur with maybe five or six other classes of PAHs.

CHAIRPERSON MACK: You can set up the cohort of smokers and that will probably be as close as can you get. If there is no more comments from the Committee, we didn't get any cards. If there is anybody in the audience who would like to make any comments -- Gary, anybody else, please say so now or forever hold your peace. Okay.

That being the case, we're ready to think about a vote. And the first way we'll do it as by addressing the issue of the class as a class of carcinogens. So I will now read the official wording for the voting protocol.

Have dibenzanthracenes been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer?

Everybody would agree with that statement raise your hand, please.

(Hands raised)

CHAIRPERSON MACK: All those voting no, please raise your hand.

And those abstaining, please raise their hand.

(Hand raised.)

COMMITTEE MEMBER LANDOLPH: For that question, I'd like you to ask a more specific question.

CHAIRPERSON MACK: So you're abstaining on this question?

COMMITTEE MEMBER LANDOLPH: On the question of the class. We don't have any data.

CHAIRPERSON MACK: We have to record the responses. So as I see it we have one, two -- six yeses and zero nos and one abstention; correct?

All right. Then we go to -- so that gives us a positive vote from the Committee; is that correct?

CHIEF COUNSEL MONAHAN-CUMMINGS: That's correct for the class, the group. If Dr. Landolph --

CHAIRPERSON MACK: We can still go onto vote --

CHIEF COUNSEL MONAHAN-CUMMINGS: You could list them separately as well. But they would be essentially subsumed. They would be double listed. But the one is already listed, so it wouldn't make a huge difference.

CHAIRPERSON MACK: So this is to some extent an academic procedure. But we'll do it anyway.

COMMITTEE MEMBER LANDOLPH: I don't think it is. I think it's a data-driven procedure. I'm happy to vote on two separate --

CHAIRPERSON MACK: Legislatively academic. Legally academic.

COMMITTEE MEMBER LANDOLPH: I just think for the record, I don't think --

CHAIRPERSON MACK: Now let's ask the question:

Has dibenz(ac)anthracene been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer?

All those in favor of that proposal, raise their hand.

(Hands raised)

CHAIRPERSON MACK: Note so that's a unanimous judgment.

Having done that, let's go to the other one.

Has dibenz(aj)anthracene been clearly shown through scientifically valid testing and according to generally accepted principle to cause cancer?

All those accepting that proposition, please raise their hand.

(Hands raised)

CHAIRPERSON MACK: That's unanimous as well. Okay. So we like the listing both the individual

compounds and the class.

COMMITTEE MEMBER EASTMOND: As a clarification, this may come to legal staff. Are there other compounds that you would consider members of the class that aren't, in addition to these three?

CHAIRPERSON MACK: Well, in theory, there is an hj, isn't there?

COMMITTEE MEMBER EASTMOND: You could get some -- that's what I wondered about, is where there is no data at all.

DR. SANDY: There are no other isomers that are dibenzanthracenes.

CHAIRPERSON MACK: Does anybody know why there isn't hj?

DR. SANDY: We have ac, aj, and ah. And if you look at the structure of --

CHAIRPERSON MACK: You can't have the two on the bottom.

DR. WONG: Put slide two up.

COMMITTEE MEMBER EASTMOND: Technically, you could have a BI, but that would be called a different name.

DR. TSAI: Based on the IUPAC, International Union of Pure -- that in charge of the naming scheme, if you have different -- you could technically have

different. But if you flip it over, it's the same thing. So these three are the possible combination.

For example, if you have five benzene rings in a linear formation, you don't call it dibenzanthracene. Because the IPAC, they have a list of naming scheme based on the priority on their list. So these are the only three isomers possible for the dibenzanthracenes.

CHAIRPERSON MACK: Thank you. Let's go to nitrosomethyl.

COMMITTEE MEMBER EASTMOND: We're really dealing with unsubstituted dibenzanthracenes, because there are other members of the class which will have --

CHAIRPERSON MACK: Correct. You nailed them down both ways. Nobody can sneak out.

Okay, Martha.

DR. SANDY: Some introductory remarks for the next chemical.

Back in the same meeting in 2011, the CIC was asked to rank the group of chemicals called the N-Nitrosomethyl-n-Alkylamines also known as N-methyl-n-nitroso-1-alkylamines. And we brought several individual alkymines within that group to the Committee. And the Committee ranked them as high priority for selection and HID development.

In 2013, OEHHA selected the group and the

individual isomers for hazard identification preparation, and we announced that we were calling for relevant information from the public on those and we did not receive anything.

I'll turn it over to Dr. Wong, and she will introduce the staff who will be making the presentation.

DR. WONG: I would like to introduce the staff presenting in the order of presentation, Dr. Karin Ricker and Dr. Kate Li. They will present the evidence of the carcinogenicity of N-Nitrosomethyl-n-Alkylamines.

(Thereupon an overhead presentation was presented as follows.)

DR. RICKER: Thank you, Dr. Wong.

We are presenting evidence on the carcinogenicity of the chemical group, N-nitrosomethyl-alkylamines. We will refer to this group as NMAs.

The information presented here was developed to assist the Cancer Identification Committee in its deliberation on whether or not NMAs as a group, or individual chemicals within the group, should be added to the Proposition 65 list as causing cancer.

--o0o--

DR. RICKER: We will start this presentation with background information on chemistry, use & occurrence of

NMAs.

And follow with evidence on carcinogenicity from animal studies, genotoxicity, pharmacokinetics, and structure activity relationships.

We will also present information on possible mechanisms of action, review by other agencies, and conclude the presentation with a data summary.

In the interest of time, the data presented today are very condensed. A much more detailed summary of the findings is contained in the HID that was presented to the Committee.

--o0o--

DR. RICKER: The basic core structure of an NMA is shown in this slide here in the upper left corner. NMAs contain a nitroso group, shown here in the red circle. There is a second nitrogen to which a methyl and a linear alkyl group are attached. The smallest attached alkyl group is a methyl group. The carbon atoms closest to the nitrogen are referred to as alpha carbons.

Individual structures of NMAs reviewed in the HID are presented in Table 1 of the actual HID document and I will show you a list in a moment.

NMAs have been detected in personal care products such as shampoos or conditioners; and household cleaning

agents such as liquid dishwashing detergent.

NMAs are not intentionally added to these products but can form from fatty amine oxide precursors which are added as emulsifiers, detergents, or thickeners; they can also form from preservatives like bronopol and bronidox.

--o0o--

DR. RICKER: Here is a list of NMAs for which we found data and which were reviewed in the HID. As you can see, the first two members in this group are already on the Prop. 65 list for causing cancer. The other NMAs are not on the Prop. 65 list and are brought to the Committee today for their evaluation.

Because the names of the individual NMAs are very lengthy, we will use an abbreviation. For example, we will refer to N-nitrosomethyl-n-butylamine as NMA C4 based on the individual NMAs particular alkyl chain length. These individual abbreviations are shown here in the right column on this slide.

--o0o--

DR. RICKER: Here is a brief outline of what we will present today:

No human epidemiology studies were identified but we reviewed over 90 animal studies. The results of these animal studies will be presented in the next few slides.

You will hear additional evidence from genotoxicity studies, pharmacokinetics, and metabolism studies, as well as structure activity comparisons.

I am now turning the presentation of the animal study data over to my colleague, Dr. Kate Li.

--o0o--

DR. LI: Animal carcinicity studies for NMAs C3 through C14 were identified in four animal species.

Here are the summary table of these studies listed by number of strains, routes of exposure, and number of positive studies

For example, in rats, assays were conducted in five strains, namely, Fisher, SD, Wistar, BD rats and Japanese strain Donyu rats. Animals were exposed to NMAs by seven routes. These routes include subcutaneous, dw, gavage, ip, iv, intramuscular, and transplacental. The details are all in the HID document.

There is one guinea pig study by the gavage route. Due to time constrains, I will focus on the rat, hamster, and mouse studies in this presentation.

--o0o--

DR. LI: A range of dose levels, exposure durations, and study durations has been investigated in bioassays.

There are also limitations in some of the studies designs.

Most studies have small group sizes. Some studies include multiple dose groups. Others include one dose group.

Although many studies have less than lifetime exposure and study durations, carcinogenic effects were observed.

Several studies lack concurrent controls, but each NMA has some studies that included concurrent controls in this report.

Overall, some NMAs have been tested in different laboratories, with similar tumor findings reported across studies.

Treatment-related tumors often observed at multiple target sites different routes, across species, strain, sex, or age at exposure.

As see in the next slide --

--o0o--

DR. LI: -- we listed the major tumor sites based on number of positive studies in rats, hamsters and mice. These are nasal cavity, tongue, larynx, trachea, bronchial tract, lung, esophagus, forestomach, liver, and bladder.

In the next 3 slides, I will show you the summarized findings in these three species.

--o0o--

DR. LI: In rats, carcinicity studies were reported for all eleven NMAs. This table shows positive studies in different tumor sites from left to right by individual NMA from C3 through C14.

Each positive study is defined as either significantly increases in tumor incidences comparing to controls or the occurrence of rare tumors.

For NMA C3 or nitrosomethyl propylamine, nasal cavity, esophegus, and liver tumors were reported in both males and females. Tongue and stomach were observed in females only.

The MF here as we see in C5 indicates that the results were reported as male and female combined.

I won’t go through each chemical here, and they are detailed in the document.

Overall, each NMA induced tumors in multiple sites.

The blank boxes are those we don’t have positive data or not being tested.

--o0o--

DR. LI: In hamsters, carcinogenicity studies data were available for seven chemicals: Namely, NMA C3

to C8, and C12.

Here, LTB is the short name for larynx/thrachea/ branchio tumars.

As you can see, each NMA induced tumors in multiple sites in hamsters.

--o0o--

DR. LI: In mice, carcinogenicity studies were reported in NMAs C3 and C5 in multiple mouse strains.

Studies here in C3 exposure induce nasal cavity, LTB, lung and liver tumors in females.

--o0o--

DR. LI: As I just show you, each chemical induced tumors in multiple sites. For NMAs C3 through C8, and C12, carcinogenicity were investigated in more than one species.

This slide lists the rare tumors in each species. For the tumors displayed, many are rare tumors by sites. All types of nasal tumors are rare in rats, hamsters, and mice.

I will also point out here that some are rare tumor types. For example, in rats, cholangiocarcinomas of liver are rare. In hamsters, hepatocellular and cholangiocellular carcinomas, hemangiomas and hemangiosarcomas of liver are rare tumor.

--o0o--

DR. LI: This is a grand summary table to show you the major tumor findings by sites in rats, hamsters, mice. When exposed to the eleven individual NMAs we are presenting here and the two Prop. 65 carcinogens, C1 and C2 on the top rows of the table.

Most target tumor sites are rare. Here, they are highlighted by the yellow background. Esophagus tumors in hamsters were reported as infrequent based on the author's description, so we using stripe patterns.

And for other tumors, we used the gray background.

Increases in rare tumor incidence are checked as X, when increases of tumor incidence is statistically significant, use X*.

For studies that doesn't have concurrent controls but with tumor incidence more than 90%, we use X1.

NT indicate for not tested. Blank boxes are the ones that there is no positive control data, either not significant or negative data.

The overall take-home message is that many common tumor sites were observed across species and chemicals.

Now I will turn to Dr. Ricker to present you the other relevant data.

--o0o--

DR. RICKER: Thank you, Kate.

I would like to turn now to genotoxicity, followed by metabolism studies and structure activity comparisons.

Briefly, evidence for genotoxicity stems from bacterial and mammalian mutagenicity tests as well as DNA adduct studies. All NMAs tested are mutagenic in bacterial assays; this includes NMA C1-C4, and NMA C6-C12. No data were found for NMA C5 and C 14.

A subset of NMAs were tested in mammalian mutagenicity tests. NMA C1, 2, 3 were positive in these tests.

All NMAs tested also form DNA adducts as shown in in vivo studies with rats.

--o0o--

DR. RICKER: Another piece of evidence comes from the findings of ADME studies, as well as from studies conducted with tissue preparations or microsomal fractions from humans, rats, mice, hamster, and guinea pigs.

--o0o--

DR. RICKER: Here are some of the key findings:

In rats, ADME studies show that NMAs are rapidly absorbed, distributed and excreted within 24 hours following oral dosing or ip injections.

In humans, NMAs are absorbed to a limited extent in in vitro experiments using human skin.

NMAs require metabolic activation by Cyp P 450 enzymes, and a key step in this process is the hydroxylation of the alpha carbons. P 450 oxidation leads to the formation of various oxidation products and nitrite. We will see this in more detail on the next slides.

Results from multiple metabolism studies also show that metabolism is similar across species and similar across all NMAs investigated.

Another important finding is the fact that several common metabolites formed are carcinogenic and genotoxic.

--o0o--

DR. RICKER: Here we have listed the common carcinogenic and genotoxic metabolites that have been observed across species and across individual NMAs.

Two compounds are known carcinogens, namely formaldehyde and N nitrososarcosine. The other three compounds induce tumors in animals.

--o0o--

DR. RICKER: This slide shows in more detail the routes of proposed NMA metabolism with NMA C4 as an example. Let’s focus first on the middle and right side of the slide. NMA C4 is initially hydroxylated by P450 enzymes at the alpha carbon of either methyl or alkyl

group. This step leads to the formation of a hydroxy methyl alkyl nitrosamine followed by the formation of a mono-N-alkyl nitrosamine and aldehydes, in this case butyraldehyde and formaldehyde, a known carcinogen, shown here in the red circle. The mono alkyl nitrosamines spontaneously decompose to form diazonium ions which then can alkylate DNA.

Now we move to the left side of the slide. P 450 enzymes can also oxidize NMAs at the non-alpha carbon of the alkyl chain. This leads to a variety of hydroxylated products, including 4-HO nitrosomethylbutylamine, which is mutagenic and carcinogenic in animals.

The other mutagenic and carcinogenic metabolites are formed further downstream and include N-nitrosomethyl-3 carboxypropylamine. MOP, and the known carcinogen N nitrososarcosine circled in red here.

--o0o--

DR. RICKER: We are now turning to structure activity comparisons. This overview slide shows the structures of chemicals in the first column and their cancer classification in the other two columns. In the first row, we have the structures of NMAs that you are considering today, the NMAs.

In the next row we have the structures of NMAs that are already listed, NMA C1 and C2.

The last three rows show individual chemicals of a group that is structurally very similar to NMAs. This group is referred to as N-nitrosodialkylamines. The selected members are: N nitroso di ethyl, di propyl, and di-butyl amine.

All chemicals share the nitroso group as well as other structural similarities highlighted here in red; they share linear alkyl groups, which can be symmetric or non-symmetric.

As pointed out here, all these chemicals are known carcinogens on the Prop. 65 list, and they have been classified as carcinogens by several authoritative bodies.

--o0o--

DR. RICKER: This slide summarizes some of the results of the structure activity comparison in rats.

The top row across lists tumor sites. This first column on the left here lists individual NMA chemicals and the comparator chemicals. The letter X designates the tumor sites observed with these individual chemicals, and the letter R denotes rare tumors.

Briefly, we see a pattern of multiple tumor sites for each chemical, many common tumor sites shared amongst structurally similar chemicals, and many rare tumor sites.

--o0o--

DR. RICKER: This slide is very similar to the

last slide and shows tumor sites in hamster. Again, we see a pattern of multiple tumor sites for each chemical, many common tumor sites shared amongst structurally similar chemicals, and we have many rare tumor sites.

--o0o--

DR. RICKER: A review of available data suggests that NMAs act via genotoxic mechanisms.

This is supported by the fact that NMAs are mutagenic in bacterial cells, and several NMAs are mutagenic in mammalian cells. Furthermore, CYP 450 activation of NMAs is required which in turn can result in the formation of reactive compounds like alkyl diazonium ions with subsequent possible alkylation of DNA.

Lastly, we know from metabolism studies that carcinogenic and genotoxic metabolites are formed.

--o0o--

DR. RICKER: Here we list briefly the review of NMAs by other agencies. The only NMAs that have been classified by other agencies are NMA C1 and C2. All other NMAs have not been classified.

--o0o--

DR. RICKER: And we are concluding our presentation with a couple summary slides here. We had a positive evidence from over 90 animal studies. Most

studies have small group sizes with a range of dose levels, exposure, and study durations. Several studies lack concurrent controls. But NMAs have been tested in different laboratories with similar tumor findings reported across studies. Tumors were observed with all NMAs tested. Positive tumor findings were found with multiple exposure routes. We had significant increases in tumors in multiple species, strains, and multiple sites. Many rare tumor sites and tumor types were observed, and common tumor sizes across species and NMAs.

--o0o--

DR. RICKER: Furthermore, we had positive genotoxicity studies, the formation of DNA adducts in vivo, and similar metabolism across chemicals and species, including the formation of carcinogenic and genotoxic metabolites. NMAs share common tumor sites with structurally similar carcinogens.

And with that, I conclude our presentation. Thank you.

CHAIRPERSON MACK: Thank you.

Does anybody on the Committee have any questions for the staff? Yes, Dr. Bush.

COMMITTEE MEMBER BUSH: So in looking at the data and particularly Table 2, which is a big table of all the animal bioassays, C5 was particularly prominent in that in

all of these studies and all of these bioassays. In fact, nearly half the table is data from C5. And I sifted through the literature, and I couldn't actually find any information of why that's the case. Do you have any insight there?

DR. RICKER: I can take a stab at this.

C5 is used as a positive control in many bioassays just because it causes distinct esophagus tumors. That's why we have a multitude of C5 and a hodgepodge of data on some of the other chemicals.

COMMITTEE MEMBER BUSH: And I was leading with that. So there was nothing in any of other studies that would suggest or indicate that there was any of these other NMAs that may be being used as a positive control in any way?

DR. RICKER: We didn't find any.

DR. LI: There are multiple studies for C3, C4, and C12. But we don't find -- C5 in many reports, it's more like the title of the report study for some other chemicals. And then within, the report we have a positive control, which is C5.

COMMITTEE MEMBER BUSH: Okay. Thank you.

CHAIRPERSON MACK: Shanaz.

COMMITTEE MEMBER DAIRKEE: Aren't there -- any is there any information about the levels present in personal

care products and how they compare with what's given to animals experimental studies?

DR. RICKER: I can tell you the levels that we found in literature. The detected range is between eight and 873 parts per billion. So it's fairly low.

COMMITTEE MEMBER DAIRKEE: And that's in --

DR. RICKER: In personal care products. Shampoos, conditioners. That's the range that we found was reported.

COMMITTEE MEMBER DAIRKEE: But it's a consistent exposure throughout life if you use these things.

DR. RICKER: Well, the findings -- not all products contain these NMAs. And in fact, a lot of them don't have it. So the literature we reviewed just showed where it was found and that includes North American and European products.

COMMITTEE MEMBER DAIRKEE: Thank you.

CHAIRPERSON MACK: Dr. Zhang.

COMMITTEE MEMBER ZHANG: Is there any evidence to show dose response? Seems to me I haven't seen any single table that's dose response or any study has tested on different dose. But I thought I heard when you present you were saying some study has multiple doses. But from what I read is multiple doses for different compound. But same compound, did we see dose response. Let's say

genotoxicity in vitro studies, did you see any dose response? I haven't see anything there listed on the table.

DR. LI: I can answer the in vivo bioassays portion. There are some studies they have test multiple doses. Some of them do have the dose response at certain tumor sites, which you will be able to see in when we present in Table 25 through Table 31 for -- I can point you like page 57, which is Table 31, C12 chemical in male hamsters has multiple doses. We have the first dose it's our controls. Then we have three stars, means it's significant in trend test. We have the other dose for urinary tract tumors, we have other low, mid, high dose which we can see just the dose response with statistically significant increases of dose.

COMMITTEE MEMBER ZHANG: Okay. Sorry I missed it. It's the same thing when you have stars in the zero --

DR. LI: Zero is for trend test.

And also if you look at the exposure column of the table, we have the table in the same format for each NMA member. So in exposure column, you will be able to tell in this case low, mid, and high. And in many other studies, obvious, they only study one dose. So we don't have that. We don't have that.

CHAIRPERSON MACK: Carol was going make a comment.

CHIEF COUNSEL MONAHAN-CUMMINGS: I just wanted to address the questions that you've had, Dr. Dairkee, concerning current exposures for humans from these different chemicals. And I just want to clarify just mostly for our record that this Committee is kind of unique in that it only looks at hazard identification piece of the process. And so concern about the actual current levels of exposure to humans isn't really relevant to the decision-making process here. I know it's of interest certainly in terms of concern about current exposures, obviously. But we don't generally present a lot of information on that.

We do say this is how people might be exposed, but we don't do a lot of research on that, because it isn't relevant to the decision that you all need to make about whether or not it, in fact, causes cancer.

There is one piece of your criteria that talks about the dose that's given to an animal, for example, and whether that's somewhat relevant to the comparison to the human reactions to that dose or something. Once again, I'm not a scientist. But that's I think a different question and Dr. Mack can speak to that. But I just wanted to clarify that's why our staff don't have specific

information about current exposure levels necessarily because that's not the focus of the information for --

CHAIRPERSON MACK: My understanding is I don't think it's relevant to the issues that we have to discuss because the more commonly the exposure, the more likely it would be that there would be better studies or more studies of a given. So establishing that something is very common in the absence of a lot of high quality studies is a pertinent scientific observations.

CHIEF COUNSEL MONAHAN-CUMMINGS: Sure. I understand that.

CHAIRPERSON MACK: David.

COMMITTEE MEMBER EASTMOND: This is kind of a general question. But so when we're talking about a group, right now you have these are N alkyl isomers that go between C2 and C14. If we had a compound that was a C15, would it fall within this group or not? Because I mean, that's -- as far as the listing, that becomes important. You know, is the listing restricted to the chemicals we actually have seen data for or does this even go beyond that?

DR. SANDY: So if you are asked and vote on listing this group of chemicals, NMAs, I'll use the shorthand term as a group, it would then cover any of those NMAs, including NMA C13, which we have no data for

or 15.

COMMITTEE MEMBER EASTMOND: But it could keep going on.

CHAIRPERSON MACK: Or 25.

DR. SANDY: Yes.

COMMITTEE MEMBER LANDOLPH: That's exactly the point I was trying to address last time. I think my predilection would be they would be very precise in those compounds that we have data for. I don't want to get trapped into something that we don't have data on.

CHAIRPERSON MACK: Are there any other comments or questions for the staff?

COMMITTEE MEMBER ZHANG: So seems like you answer one of my question. So C13 actually is a compound C13, just we don't have data on C13.

DR. SANDY: Theoretically, there is a compound called C13 and a compound called C20 and C30. We don't have data on them.

I would add as we brought these to you for prioritization -- chemical groups -- and you asked us to do that, you might also think about the process 20 years ago or more when people, not just the CIC, but other groups were evaluating PCBs and dioxins and other groups, did they require data on 209 congeners. It's all up to you and your decision, but I just put that out there as

well.

CHAIRPERSON MACK: David.

COMMITTEE MEMBER EASTMOND: I have this question. I'll see you what you think. You've been thinking about this a lot longer than I have.

One of the strongest evidence for this is these all -- all of this class can be metabolized through this alpha carbon to the same reactive intermediate. And so therefore, you would expect real commonality of tumor sites, but you're showing a lot of them are very similar. But there are differences.

Any speculation? I mean, I have some ideas. Have you thought about why you would see different tumor types from this class?

DR. RICKER: I'm taking a wild guess. I don't know the answer. But you know, we had so many different animal species. And within rats, we had several types of varieties and also the mode of administration with different routes. I'm not sure there is some -- in some cases, the pair matches up. I think we had some DNA alkylation matches up with tumors and these kinds of things. But it doesn't always sort of pattern isn't as clear.

CHAIRPERSON MACK: I'm not a chemist or toxicologist, so I'll turn it back on you. Wouldn't you

expect to see some difference in the distribution of a compound that has a completely different shape and a completely different size?

COMMITTEE MEMBER EASTMOND: Yes. The other aspect is there are other types of reactive metabolites formed. So the one common goes all the way through it, but there are others as well. You can expect differences. But I just thought -- you've been thinking about this a lot longer than I have. That was the thing that jumped out at me. It's not surprising, but it was something I was going to get their perspective on.

CHAIRPERSON MACK: But our job is not to decide what kind of cancer. Our job is to decide whether or not it's carcinogenic; right?

COMMITTEE MEMBER EASTMOND: Yeah.

CHAIRPERSON MACK: So if there are no more questions for the staff, let's go to Dr. Bush.

COMMITTEE MEMBER BUSH: All right. Well, again, I want to start off by thanking the team for putting together this data. The summary tables were very useful and I think indicate some compelling evidence.

And when I look at the evidence of the carcinogenicity in total, and I'm going to call them NMAs as well, as we've all seen is there is this remarkable commonality for this class.

I'll start off with the bioassays since there is no human carcinogenicity data available. Having multiple tumors from particularly and epithelial origins and showing that really a progression from benign to malignant kinds of tumor types have that pre-neoplastic precursor certainly are evidence of that common root, along with the common tumor sites that we're actually seeing.

The presence of these rare tumors and the possibility I would speculate that it may be due to the different profiles of the P450s in different cell types, but that's only speculation. That's not our job here.

When I combined the compelling animal data with the genotoxicity, the story gets more convincing for me. We are making a leap here, I guess to some extent, that most of the animal bioassays were done in a gavage kind of model. And you know, extrapolating that question to gavage in rodents to potentially topical usage in humans, and I realize that's not the mandate here, but it does from a scientific perspective beg an interesting question here. Can we actually make any connections there.

But basing my decision on primarily the bioassay data in animals, it certainly seems like there is strong evidence of carcinogenicity there. And then again combining with the genotoxicity data, I think it's well demonstrated that there is mutagenicity in a bunch of

different prokaryotic models. We're seeing both in vivo adducts, particularly methyl adducts on DNA and RNA. That actually hints to some possible epigenetic effects as well.

The shared structural similarities with other of the alkylated amines that you introduced is part of that remarkable commonality they have. The toxic, potentially carcinogenic metabolites, specifically the nitrososarcosine and formaldehyde I think again point to this common metabolism that is going on with this class of compounds.

In going through the data, I was optimistic there might be more on the dermal absorption, trying to make this leap with topical usage in humans. But there is only a few studies out there with dermal absorption and trusting they actually mix it with shampoo as a vehicle. But the metabolites and the absorption do pose a common root that you indicate before. So when I weight the evidence, I find that there is convincing for me at least that this class certainly has strong carcinogenic properties.

CHAIRPERSON MACK: Dr. Zhang.

COMMITTEE MEMBER ZHANG: I think Dr. Bush has very good job to summarize. And also the OEHHA staff did a wonderful job pulling the report together.

And also I'm very glad to hear Dr. Bush mention the epigenetic effect. But if your really see the metabolism of the NMAs and they actually do, easy ways to have DNA in the NMA, so that's actually really correct the basis epigenetic effect.

So I think for this NMAs, not only has strong carcinogenicity data studies in animal models, but also the biological plausibility like, you know, induce genotoxicity, mutagenicity, and also I think another extra information convince me is carcinogenic metabolites during the metabolism. So considering all three together, I think I'm pretty convinced.

CHAIRPERSON MACK: Any more comments from anybody with respect to what Dr. Bush and Dr. Zhang have said?

Yes.

COMMITTEE MEMBER DAIRKEE: I had a comment about the human skin absorption paper. That was like a terrible paper. So I guess I don't need to say any more.

CHAIRPERSON MACK: I guess we're ready to take a vote. We have no cards, but would anybody from the audience like to make any comments? Hearing no -- yes, ma'am.

COMMITTEE MEMBER REYNOLDS: I just wondered if my colleagues here could comment on the issue of their take on the group versus the individual discussion we had

earlier. What your feeling -- you feel like the group is convincing enough?

COMMITTEE MEMBER ZHANG: So far, NMAs if you look at the history, C1 and C2 has been listed in 80s. And now 25 years after or 25 years or more, so we had like eleven extra compounds in the group and looks still pretty convincing.

And also I think maybe now for this chemical -- for the last chemical data the QSAR look at the structure, if you look at the chemical structure similarities and doing the comparison, I think as a group I'm actually convinced because I don't think I needed to see every single compound. Even though we don't see it, but what you have you learned from the first two chemicals 25-plus years ago and now the eleven extra chemicals and consistently show carcinogenicity in the animals and mechanistic data. So I feel I don't need to see more.

COMMITTEE MEMBER REYNOLDS: Thank you.

COMMITTEE MEMBER BUSH: I would agree. Again, what struck me was the common sites, these common progressions that we were seeing in the tumor sites and tumor types. But also what we were seeing with the metabolism, too. So for those reasons, I see the class as being remarkably similar.

COMMITTEE MEMBER REYNOLDS: Thank you, both.

CHAIRPERSON MACK: I think if somebody does a very large study either in humans or animals of C25 ten years from now, we can re-visit the issue. Okay. At least that's my opinion.

So the question -- I'll now pose the question on the group basis.

Perhaps, Joe, if you have a comment. You were resisting last time.

COMMITTEE MEMBER LANDOLPH: I'm still going to do the same thing this time. I'm not real wild about making decisions based on no data. I do agree that there is a consistency from what we've seen so far. But as you get these longer hydrophobic tails, unusual chemical effects can occur. So I'm not willing to concede at this point I'm ready to vote as a group.

I'm very happy to vote on what we've seen, but not what we have not seen data on.

CHAIRPERSON MACK: I'm very reluctant to go through 15 individual chemicals.

COMMITTEE MEMBER LANDOLPH: We can just --

CHAIRPERSON MACK: I think we should take a vote on the group. See what happens. And then we can discuss what to do subsequently.

COMMITTEE MEMBER LANDOLPH: Why don't you say NMA C3 to C12 and C14. That takes ten seconds to say.

CHAIRPERSON MACK: You're free to make nominations.

COMMITTEE MEMBER EASTMOND: Having debated back in forth in my mind, arguably no matter how long that chain is, you will still get methyl diazonium ion, presumably have metabolism. That could be reactive. On the other hand, if you get an long enough tail, it would interfere with its absorption. So ultimately this may be too broad. So I wondered about trying to limit it to what the data shows.

And that isn't kind of my interpretation of the Prop. 65 language. Clearly shown through scientifically valid testing according to generally accepted principles. And that would imply to me you actually have -- there have been tests done on those compounds. I realize you have latitude because there are going to be metabolites in similar ways you get the same reactive intermediates. So that's why I've been debating back and forth on this.

CHAIRPERSON MACK: If we say C3 through 14, we'll be excluding 13. And you won't like that. It doesn't come into your --

COMMITTEE MEMBER EASTMOND: I'm okay with that.

CHAIRPERSON MACK: How about that, Joe? Are you okay with 13?

COMMITTEE MEMBER LANDOLPH: Why don't you say C3

to C12 and C14.

CHAIRPERSON MACK: All right.

COMMITTEE MEMBER ZHANG: I like to make one more comment before voting, if that's okay.

Yes, from the 013, 013 of the chemical in NMAs, so if you look at the chemical properties when the carbon chain grows, actually the chemical property -- some property get changed as well. For example, on the Table 1 listed can change. So that's also could -- I mean, I have to -- I was trained as a chemist. I have to measure the chemical property would change when the carbon chain grow.

CHAIRPERSON MACK: Dr. Zhang, would you be happy if we took a vote on C3 through C12 plus C14?

COMMITTEE MEMBER ZHANG: Yes.

CHAIRPERSON MACK: Okay.

COMMITTEE MEMBER ZHANG: I could move the entire group or subgroup.

CHAIRPERSON MACK: Let me ask Carol. Who has set this in stone?

CHIEF COUNSEL MONAHAN-CUMMINGS: Nobody has set it in stone. It's up to you and your scientific judgment how you want to approach it. You can list the group if you think that's appropriate. But if you feel and the majority of the Committee feels that you want to just vote

on the certain ones, then that's absolutely fine.

CHAIRPERSON MACK: My sense is that the minority of the Committee would feel more comfortable dealing with 2 through 12 plus 14, because that's where the data is. It doesn't mean that we couldn't have to force the staff to look at huge amounts of evidence in the future on 13, 16, and 18. But it seems pretty unlikely that would happen.

Let me put the -- may I make a straw vote on how many people would prefer to do each of the alternatives.

How many people would like to vote first on all such compounds? So even including 25 and 26 and 27?

COMMITTEE MEMBER ZHANG: Uh-huh.

CHAIRPERSON MACK: That's one.

Does anybody else?

Okay. How many people would prefer to vote on 1 through 12, plus 14. 3 through 12, plus 14.

COMMITTEE MEMBER ZHANG: I'm fine with that, too.

CHAIRPERSON MACK: We're getting a large consensus there.

So if nobody minds, I would like to amend the statement to say have N-Nitrosomethyl-n-Alkylamines 3 through 12 plus 14 -- I'm sorry -- c3 through C12 plus C14 been clearly shown through scientifically valid testing and according to generally accepted principles to cause

cancer? How many votes yes to that statement?

(Show of hands)

CHAIRPERSON MACK: Okay. We have unanimity. Isn't that nice.

All those voting no?

All those abstaining? No.

So we now announce the results, and more than four yes votes been provided to pass that statement.

I now turn it back over to you, I think. Carol, it's your stage.

CHIEF COUNSEL MONAHAN-CUMMINGS: Okay. I have to say that I'm not used to doing this part of the staff update, but I as I mentioned earlier, we're down a few staff. So Cindy used to actually pronounce all these chemicals. I'm not going to try to do that.

What you have up here on the first slide is a list of the chemicals that we have administratively listed since the last time you had a meeting in December of 2013. You can see that the majority of them are carcinogens and you can see from the chart that they were all -- what the dates were that they were listed.

The next slide shows you a number of chemicals that have actually been delisted since your last meeting, and all of them are reproductive toxicants.

Just as an explanation for this, it's not

particularly common for us to delist a chemical, and certainly not this many in one year. And so as an explanation for that, I should let you know that the federal OSHA did a pretty massive re-write of their regulations that have to do with hazard communication standards. And for occupational exposures, that directly impacted our ability to list chemicals pursuant to the OSHA regulations. So they eliminated a couple of the basis for listing the chemicals, one in particular being the list of threshold limit values that are published by the American Conference of Governmental Industrial Hygienists.

So in any event, they eliminated that group as a definitive source for identifying reproductive toxicants. A number of these chemicals -- most of them have been listed years ago. But we looked at them, and there wasn't another basis for keeping them on the list. So they went through the process of being presented to the DART Committee for possible retention on the list. And these are the ones that fell out of that process. So they have been delisted on the dates that you can see here.

Next slide.

--o0o--

CHIEF COUNSEL MONAHAN-CUMMINGS: So in terms of chemicals that are still under consideration right now, we

have three carcinogens and two developmental toxicants. These are currently in process. You can see on the right-hand column the date we had proposed the listing. We haven't made final determinations on any of these, whether we'll proceed with the listing. We're still looking at public comments. And actually on the last one, on the list, the public comment period hasn't closed yet. So these are coming up.

I don't know if you have any questions on any of the chemicals. I should remind you that hopefully you're on our list serve for our Prop. 65 activities. And I would encourage you to be on there if you aren't and make comments to the extent that you think you feel it's appropriate. You can do that as an individual Committee member. You don't have to do it as a group. Yes.

COMMITTEE MEMBER EASTMOND: So these are under consideration based on authoritative body process or what?

CHIEF COUNSEL MONAHAN-CUMMINGS: There's different basis for different ones. We've got, like the last one, for example, is a drug. And it is being proposed for listing based on what we call our formally required listing process. And that is where a government agency such as FDA requires a warning for the chemical as a carcinogen. And for in this case, they do in this package insert for this particular product, it calls it a

carcinogen. So we're required to list it basically.

COMMITTEE MEMBER EASTMOND: Probably should be listed, too.

CHIEF COUNSEL MONAHAN-CUMMINGS: The first two are authoritative bodies. So they're both authoritative bodies listing under NTP; is that correct?

DR. SANDY: First one, beta myrcene is an authoritative body under NTP and the nitrate with combination of amines and amides is an authoritative body listing are under IARC.

CHAIRPERSON MACK: That's what I wanted to know.

CHIEF COUNSEL MONAHAN-CUMMINGS: Anything else? So then --

CHAIRPERSON MACK: Litigation.

CHIEF COUNSEL MONAHAN-CUMMINGS: Your favorite subject. Mine, anyway. That's why I have a job.

So in terms of the litigation that is pending currently against our office, there is two cases that are related to cancer and two related to reproductive toxicity. And oddly enough, there is only two names for these.

So for cancer, we have a case called Syngenta versus OEHHA. We're calling that Syngenta 1. That case was filed in 2012, and it's challenging indirectly now our safe harbor level for the chemical chlorothalonil, which

is a pesticide. We're in the discovery process in that case. We have a trial date in September of 2015. And it's taking a lot of staff resources to work on that. They are not challenging the actual listing of chlorothalonil as a carcinogen. It's the level of the safe harbor they're challenging.

The other one that's related to cancer is the American Chemistry Council versus OEHHA, which we're calling ACC II. And that was filed in June of this year. It's challenging this Committee's listing of the chemical DINP last year.

Interestingly enough, this Committee has not been sued. They are not part of the lawsuit right now. They have sued us directly. But you will recall that I advised you that there is a litigation hold on your materials, so please keep those until I tell you not to.

We have a hearing on the merits in this case. Because it's a record case, that can get to trial much quicker. And that is on January the 23rd in Sacramento. You have a question?

COMMITTEE MEMBER EASTMOND: The material we have to keep is related to DINP only or everything that has been discussed?

CHIEF COUNSEL MONAHAN-CUMMINGS: It's only as to DINP at the Committee.

COMMITTEE MEMBER EASTMOND: So I can throw away this stuff and be fine?

CHIEF COUNSEL MONAHAN-CUMMINGS: This meeting?

COMMITTEE MEMBER EASTMOND: Yeah.

CHIEF COUNSEL MONAHAN-CUMMINGS: This meeting is not covered by anything that I'm aware of now. Throw it away quickly. So you didn't hear that from me and it's not on the record.

Anyway, so we have two other cases that are pending that have to do with reproductive or developmental toxicants. As you may recall, the American Chemistry Council had sued us in 2013 regarding the brief listing of BPA as a developmental toxicant under Prop. 65. We are -- that was an authoritative body listing that followed a proposed listing by the DART Committee. They declined to list, but we listed under the authoritative body process. And we have a hearing on the merits of their challenge to that listing next month on December the 5th. So we're hoping by that time we'll at least have a trial court decision early next year, and that will be followed by no doubt by appeals.

The other Syngenta case that we have we're calling Syngenta II was filed in April of 2014. And it has to do with the potential listing of the group of chemicals we're calling the triazines. You saw them on

the slide earlier that it's a pending listing. That case is on hold right now waiting for us to make a final decision over whether or not we'll list the chemicals. If we decide to list them, we have to give Syngenta some notice so they can do whatever they want to do to keep us from completing the listing.

There's other cases that I believe are cued up, but I can't tell you right now when they're going to land.

In terms of other activities for OEHHA, normally we update you on our safe harbors, which are the levels that we set to give compliance assistance to businesses so they know whether or not a warning is required for a particular exposure. We have not adopted any safe harbors this year, although we have some that we have been looking at, given the litigation currently pending on our safe harbor for chlorothalonil and also other resource issues we have not proposed any safe harbors. And we don't have any that are imminent to be proposed.

In terms of regulations, I did want to point out a couple things out. We did complete a regulatory change to the 60-day notice requirements for Prop. 65 that have to do with notice of violation. We completed the process for the Committee qualifications regulation. I mentioned that to you all last time or time before. And you'll be happy to know you all meet the Committee qualifications

that are in our regulations.

We have almost completed the regulatory process for establishing the criteria for listing chemicals under what we call the Labor Code listing mechanism. That's the one that I mentioned earlier under CalOSHA or federal OSHA. And we're involved in two rather large regulatory actions right now that have to do with the regulations that apply to how to provide a warning when one is required. And also developing a regulation for a website that would be specific to chemicals where warnings are being provided. And it would give people a lot more information. And hopefully, it would be structured in a way that would be useful to members of the public that go to our website for information when they see a sign or label on a product.

So you might be seeing some stuff about that in the future. And again, you know, if you individually want to comment on any of our public actions, you're absolutely welcome to do that. And we appreciate any input that you give us. I think that's it.

CHAIRPERSON MACK: Coming back to you now.

DEPUTY DIRECTOR ZEISE: All right. So I'll summarize the Committee's actions today.

The Committee listed Dibenzanthracenes as a group with six yes votes, zero no votes, and one abstention.

The Committee also separately listed Dibenz ac anthracene unanimously with seven yeses. And also separately listed Dibenz aj anthracene with seven yeses.

The Committee also listed N-Nitrosomethyl-N-Alkylamines for the alkylamines with chain length 3 through 12 and with chain length 14. And that was listed unanimously with seven yes votes and zero nos.

So now I'd like to give some thank yous and first to thank the Committee for taking time out of your busy schedules to donate your expertise and your efforts to the State of California and to the Prop. 65 process. We really sincerely thank you for all of the effort.

CHAIRPERSON MACK: Of course.

DEPUTY DIRECTOR ZEISE: And I'd also like to thank the public for coming to the meeting, for your interest in Proposition 65 both here in the room and on the web. Thank you. And also to the staff who really worked tirelessly to put together these excellent materials. I think we can all agree on that.

CHAIRPERSON MACK: We'd like to really thank the staff for doing a terrific job.

DEPUTY DIRECTOR ZEISE: And I'd always like to thank Monet Vela who really stepped up to the plate to help us out during this time. We were down on Proposition

65 implementation staff. So thank you, Monet. And turn it back to you.

CHAIRPERSON MACK: Well, I'm going to say thank you for a nice day. We're finished. We can go have lunch.

(Whereupon the Committee adjourned at 12:39 PM)

CERTIFICATE OF REPORTER

I, TIFFANY C. KRAFT, a Certified Shorthand Reporter of the State of California, and Registered Professional Reporter, do hereby certify:

That I am a disinterested person herein; that the foregoing hearing was reported in shorthand by me, Tiffany C. Kraft, a Certified Shorthand Reporter of the State of California, and thereafter transcribed into typewriting.

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

IN WITNESS WHEREOF, I have hereunto set my hand this 10th day of December, 2014.

TIFFANY C. KRAFT, CSR, RPR
Certified Shorthand Reporter
License No. 12277