MEETING

STATE OF CALIFORNIA

ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

PROPOSITION 65

CARCINOGEN IDENTIFICATION COMMITTEE

JOE SERNA JR.

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A P P E A R A N C E S

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Peggy Reynolds, Ph.D.

Luoping Zhang, Ph.D.

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Mr. Allan Hirsch, Chief Deputy Director

Dr. Melanie Marty, Acting Deputy Director, Scientific Affairs

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

Ms. Michelle Robinson, Proposition 65 Implementation

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

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

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

STAFF:

Dr. Patty Wong, Pesticide and Environmental Toxicology Branch

ALSO PRESENT:

Dr. Don Bjerke, Procter & Gamble, Personal Care Products Council

Ms. Carol Brophy, Sedgwick, LLP representing Big Lots

Dr. Jessica LaRocca, Dow AgroSciences

Dr. Gagik Melikyan, California State University, Northridge

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P R O C E E D I N G S

ACTING DIRECTOR ZEISE: Okay. Welcome, everyone. And to people in the web studio handling the meeting, we're starting now. So I'd like to welcome everyone to the meeting of the Carcinogen Identification Committee. I'd like to welcome people in the audience and also those participating on the webcast.

Since this web -- this is being webcast, please be sure to speak clearly into your microphones, so that everything is picked up. It's also being transcribed.

Just a few comments on meeting logistics. There's drinking fountains and restrooms located -- you go through the back door and you turn left down the hall and walk quite a ways and they're located on the right.

In the event of an emergency, say a fire alarm, we need to evacuate the room. So if you would leave by the lighted exit doors, take the steps down, go outside and there's a meeting place across the street.

We have -- as I said the meeting is being transcribed, and we have our transcriber here. And we'll be taking breaks to give him a break, but he is able to transcribe quite a while before breaks.

Okay. So now I'll introduce the members of the CIC. To my immediate right is Dr. Thomas Mack from the University of Southern California School of Medicine; to

his right is Dr. Joe Landolph, Associate Professor University of Southern California; to his right is Dr. Peggy Reynolds, Senior Research Scientist at the Cancer Prevention Institute of California, consulting professor at Stanford University School of Medicine, Department of Health Research and Policy; and to her right is Dr. Jason Bush, Associate Professor, California State University, Fresno.

Then to my left is Dr. David Eastmond, Professor and Chair, Department of Cell Biology and Neuroscience, University of California, Riverside; then Dr. Shanaz Dairkee, Senior Scientist, California Pacific Medical Center; and at the end of the table to her left is Dr. Luoping Zhang, Associate Adjunct Professor, School of Public Health, UC Berkeley.

So I'm Lauren Zeise. I'm Acting Director of the Office of Environmental Health Hazard Assessment, and I'll introduce my staff. Dr. Carol Monahan who is our Chief Counsel at OEHHA; Dr. Melanie Marty whose hand is up at the back of the room, Acting Deputy Director for Scientific Affairs. Seated next to her is Allan Hirsch who is our Chief Deputy Director. Up here at the table, Dr. Martha Sandy, the Branch Chief of the Reproductive and Cancer Hazard Assessment Section; Dr. Patty Wong sitting next to her with her raised hands, is a senior

toxicologist with OEHHA; Karin Ricker -- Dr. Karin Ricker who is a staff toxicologist within Martha's branch. Meng Sun who is also a toxicologist within RCHAB; and finally, Gwen Osborne who's an associate toxicologist within RCHAB.

So I'm going to ask Carol to make some introductory remarks.

CHIEF COUNSEL MONAHAN-CUMMINGS: Good morning. Did you introduce Esther and --

ACTING DIRECTOR ZEISE: Okay. So from the Proposition 65 Implementation Office, Esther --

CHIEF COUNSEL MONAHAN-CUMMINGS: Barajas-Ochoa and Michelle Robinson, and Monet Vela. Is Monet here? I don't see her. Monet is in the back of the room. And then Julian Leichty, if you could raise your hand.

Okay. Great. Thanks.

CHIEF COUNSEL MONAHAN-CUMMINGS: Good morning. My name is Carol Monahan-Cummings. And I know all of you have served on the Committee for a while now, but I always try and give some opening remarks since we don't meet that often, and just a few reminders before we get started.

First, I'd like to remind you that you have criteria for listing chemicals that was adopted by the Committee many years ago that is always provided to you prior to the meeting and is currently in your binders as well that you can use to guide your decisions on the

either the retaining a chemical on the list or adding a chemical to the list or removing it.

Your listing decision should be based on that criteria, although it is very broad and provides you latitude in applying your scientific judgment. You should not use as criteria consideration of future impacts of the listing, for example, whether or not a warning might be required for a particular exposure. That issue is dealt with separately by OEHHA and from time to time through the courts.

The clearly shown standard, which is -- that you'll hear a lot about today and is also provided to you before you make your decision is a scientific judgment call and not a legal standard of proof. Your Committee can decide to list a chemical based on animal evidence only. The chemical need not have been shown to be a human carcinogen, and you need not determine whether or not the current human exposures to the chemical are sufficiently high to cause cancer.

The members of this Committee are appointed to the Committee by the Governor, because of your scientific expertise, and you're considered the State's qualified experts for that purpose. There's no need for you to feel compelled to go outside of that charge.

In the event that you feel that you have

insufficient information or you need more time to think or discuss the issues that are presented today, there is no requirement that you make a decision on any of the chemicals that are in front of you today. You can defer that decision to a later time.

I know that that the -- at least one of the chemicals that you're considering today is the subject of some litigation currently. And you may hear from attorneys that are involved in that litigation today. And I just again wanted to remind you that this Committee's charge is to consider scientific evidence not make legal conclusions, and not determine whether or not the existing listing of any chemicals are legally valid.

So with that, do any of the Committee members have questions for me?

Please feel free to ask any questions as you go along. Thank you.

ACTING DIRECTOR ZEISE: Okay. I'll turn the meeting over to Dr. Mack.

CHAIRPERSON MACK: I'm going to begin -- oh, there it is. I thought the light was on. It's not on.

(Laughter.)

CHAIRPERSON MACK: Okay. I'm going to say some of the things a little more rudely than Carol did. You're looking at a bunch of drudges who were willing to come on

behalf of the Governor to decide what was scientifically valid evidence and make sure that we follow generally accepted scientific principles, but that's all we're here for. It's biologic issues.

We have nothing to do with a lot of things, and I'm going to just tick some of them off. We have nothing to do with regulation. We have nothing to do with dose, except insofar as it my be useful in terms of dose response. We have nothing to do with ethics. We have nothing to do with law. This is not a courtroom. We are strictly here to judge scientific evidence.

And just a couple of other things to nail down. We're judging whether or not certain exposures cause cancer. Cause is a word that can be screwed up in many different ways. We're not predicting. We're not interested in prediction. We're not interested in association. We're only interested in relationships which, if you took away the exposure, there would be less cancer. And that's basically the definition of cause.

In terms of cancer, there's a little more difficulty. We're certainly not interested in benign tumors. We're interested in cancer by the conventional view, which usually, to most of us, I think, means invasive neoplasm, but we're a little different than some of the other bodies that make these kinds of decisions,

because in the wisdom of the people that wrote the proposal, they did not say that we're judging the causes of human cancer. We're judging the causes of cancer.

Now, it's up to us to decide whether or not a given piece of animal evidence or any other kind of evidence is -- relates to cancer.

And with that, I think we'll go ahead.

We normally take individuals who'd like to make comments, and we appreciate their contributions, to go for five minutes or so. But in this case, we've had four requests for longer periods of time, and the people that looked at the evidence that they wish to present have judged it as worthy of more discussion and more time, so we're going to have three individuals -- or three groups, give us 15 minutes of evidence on the first one, and one give us 15 minutes of evidence on the second one. When I say three, three individual sets of 15 minutes.

Now, that's going to lake a lot of time, and we're not very patient. So you might find us wriggling in our seats, but we'll do our best. And we certainly want a transparent process.

So with that, we go to -- shall we go ahead?

ACTING DIRECTOR ZEISE: (Nods head.)

CHAIRPERSON MACK: To consideration of diaminotoluenes and in mixed, and a reconsideration of

what is now a listing of diaminotoluenes mixed. And I think we'll start with you folks.

(Thereupon an overhead presentation was presented as follows.)

DR. SANDY: This is Martha Sandy. And thank you, Dr. Mack. So we'll be hearing a presentation by Dr. Karin Ricker and Dr. Meng Sun on diaminotoluenes.

ACTING DIRECTOR ZEISE: Martha, as you're getting set up, I just wanted to let the people in the audience know, if there's anyone that wants to fill out a blue card for speaking, be sure to do that, and give -- bring it to one of our folks over here. Thanks.

Okay. Go ahead.

DR. RICKER: So I'll start. Okay. Can everyone hear?

Good morning. The presentation today is on the carcinogenicity of diaminotoluenes, diaminotoluene mixed are chemicals listed as causing cancer and are under review by the CIC.

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DR. RICKER: Here's some background information on the formal identification of diaminotoluene mixed, and the reason why we brought these chemicals to the CIC today.

In 1988, U.S. EPA formally identified

diaminotoluene mixed as a Group B2 --

COMMITTEE MEMBER EASTMOND: Pull the microphone a little closer. When you look up it --

DR. RICKER: Okay. I feel like I'm eating it. Sorry.

Okay. Is that better?

So in 1988, U.S. EPA formally a identified diaminotoluene mixed as a group B2, a probable human carcinogen. In its formal identification, EPA noted that the evidence from animal studies was sufficient, that the hazard ranking applied to all isomers of diaminotoluene and was based on the carcinogenic properties of the 2,4-isomer.

In 1990, the State of California listed diaminotoluene mixed on the Proposition 65 list by the authoritative bodies mechanism. Last October, OEHHA received a petition from Big Lots stores asking for a reconsideration of the listing of diaminotoluene mixed.

We wish to point out that in addition to diaminotoluene mixed, the 2,4-diaminotoluene isomer is also listed separately under Proposition 65 list as causing cancer as noted here in the last bullet. This isomer was added by the State's qualified expert mechanism in 1988.

Today, the CIC is being asked to make a decision

if diaminotoluene mixed shall remain on the list. The CIC is also asked to determine whether or not diaminotoluenes as a group or any of the five individual isomers not currently listed should be added to the list.

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DR. RICKER: We're now coming to the more technical part of the presentation. We will begin with background information on chemical identity, use, and exposure, followed by carcinogenicity studies in animals. No suitable human epidemiology studies were identified as the studies were confounded. However, the human studies are discussed in Appendix A of the document that was submitted to the Committee and that is posted on our website.

We will also present other relevant data, which include pharmacokinetics, genotoxicity, structure activity relationships, and other information.

In the interests of time, the data presented today are condensed. A much more detailed summary of the findings is contained in the document.

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DR. RICKER: Diaminotoluenes are synthetic aromatic amines that consist of 2 amino groups and a methyl group attached to a benzene ring. The amino groups can be attached on various positions of the ring, giving

the individual isomer its name. For example, here is the 2,6-isomer with the amino groups positioned at carbons 2 and 6 of the benzene ring.

Diaminotoluenes are produced from dinitrotoluenes either via catalytic hydrogenation or by reaction with ion and hydrochloric acid. The group of diaminotoluenes under consideration today has five members in addition to the 2,4-isomer, which is individually listed. The five isomers shown are here on this slide.

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DR. RICKER: Diaminotoluenes are used as raw materials, co-reactants, curing agents, et cetera in the production of a wide variety of industrial processes and products. The most commonly marketed isomers are the 2,4 and the 2,6-isomer, which are mainly used in the production of toluene diisocyanate the 2,5-isomer is used in hair products. The most common commercially available products are listed here under the last bullet of the slide. All commercial products contain traces of the other isomers.

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DR. RICKER: Exposure to diaminotoluenes occurs in both occupational and non-occupational settings. Exposure to 2,5-diaminotoluene occurs during application of permanent hair dyes or tints. It is estimated that

about 20 percent of the commercial hair dyes in the U.S. contain the 2,5-isomer.

Exposure to 2,4 and 2,6-diaminotoluene occurs in polyurethane foam processing plants and in plants where toluene diisocyanate is produced. 2,4 and 2,6-diaminotoluenes have been detected in the urine and plasma of industrial workers, and are used as biomarkers of exposure to toluene diisocyanate.

Individuals with certain types of breast implants are also exposed to the 2,4 and the 2,6-isomer. These breast implants have a polyurethane foam cover that can break down and release the 2,4 and the 2,6-isomer. However, these specific types of breast implants are no longer approved by U.S. FDA.

Last, but not least, consumers can also be exposed by a food. Trace amounts of the 2,4-isomer have been detected in migration tests with composite food packaging bags, including bags used for chicken wings, brown sugar, oatmeal, and rice.

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DR. RICKER: OEHHA identified animal cancer bioassays for three of the six diaminotoluene isomers, namely the 2,4, 2,5, and the 2,6. These studies were published as reports by the National Cancer Institute, and include cancer bioassays in male and female rats and mice.

No studies for the other isomers were identified.

We will present the results from the 2,5 and the 2,6-isomer, as well as a summary of findings on the 2,4 for comparison purposes. We also identified numerous studies with complex mixtures containing diaminotoluenes similar to the human epi study. These studies are of limited usefulness, and the results will not be presented here, but they are discussed in the document.

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DR. RICKER: Here is a brief summary of tumor findings for the 2,4-isomer. 2,4-diaminotoluene increased the tumor human incidences of liver tumors in both sexes of rats and mice, and by two routes diet, and subcutaneous injection. In addition to liver tumors, tumor findings include rare tumors such as bone osteosarcoma in female rats and mammary adenomas in male rats. Other tumors observed include lung and mammary tumors in mice and rats respectively.

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DR. RICKER: We are coming to the bioassays with the 2,5-isomer. OEHHA identified a 1978 NCI report with four cancer bioassays, two in male and female rats and two in male and female mice. In these studies, two concentrations of the sulfate salt of the 2,5-isomer were tested via addition to the diet. There were 50 animals

per treatment with one exception of a control group that had 25 animals. Each dose group had its own control group.

Before we report on the outcome of these studies, I would like to point out some study irregularities that were noted by NCI, as well as its advisory body, the Clearinghouse on Environmental Carcinogens. The irregularities noticed concerned animal treatments, animals were received in different shipments, they were housed in different rooms, and they were provided by different suppliers.

In terms of experimental design, a different mouse strain was used in the subchronic versus the chronic study, controls and treated groups at different starting dates, and lastly, the dosing was inadequate. There was a lack of observed mean body weight depression in the animals, which led NCI to increase the high and low dose treatments in the middle of the study. In spite of these irregularities, some positive results were observed as shown on these next two slides.

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DR. RICKER: Here are the results of tumor incidences observed in the long-term feeding study with the 2,5-isomer in rats. Concentrations tested were of 600 and 2,000 parts per million respectively and each dose

group had its own control.

Treatment related increases in tumor incidences were observed in male rats, but not in female rats. I'd like to point out that this is an updated table. OEHHA sent out an errata sheet recently correcting the denominators of the testicular tumor incidences used in the hazard identification document. The reason for the correction was that the NCI animal neoplastic data file reported that days on study as weeks of study. To fix this issue, our effective numbers were recalculated based on the correct dates. As shown here, a statistically significant increase of testicular interstitial cell tumors was observed at the high dose by pairwise comparison with the controls.

NCI discounted the interstitial cell tumors in male rats based on the historically high spontaneous incidence in male rats.

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DR. RICKER: Here are the results in mice. Two doses, 600 and 1,000 parts per million were tested. Again, each dose had its own control. There were positive tumor findings in female mice, but not in male mice. In female mice statistically significant increases of lung adenomas and combined lung adenomas and carcinomas were observed in the high dose group by pairwise comparisons

with the control.

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DR. RICKER: OEHHA also identified a 1980 NCI report for the 2,6-isomer with two studies in male and female rats, and two in male and female mice. Test groups had 50 animals each. There was one only concurrent control, and a 2,6-isomer was administered via diet. The concentrations tested in rats were 250 and 500 parts per million, and 50 and 1,000 parts per million in mice.

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DR. RICKER: Here are the findings from the rat studies. There were positive findings in male rats, but not in female rats. Tumor sites are listed here on the left. Results for control, low dose, and high dose are in the columns to the right. And statistical significance by trend is noted in the last column.

In male rats, a statistically significant dose-dependent trend in the incidences of hepatocellular adenomas and combined hepatocellular adenoma and carcinoma was observed. No tumors were observed in the controls, and hepatocellular carcinomas are rare tumors in male Fischer rats.

A statistically significant dose dependent increase was also observed in pancreatic islet cell adenomas in males with an incidence in the high dose group

of 9.3 percent. Pancreatic islet cell adenomas are benign tumors with a spontaneous incidence of 3.5 to 4 percent in male Fischer rats.

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DR. RICKER: We're coming to the results of studies in mice treated with the 2,6-isomer. No treatment related tumors were observed in male mice, but there were some tumor findings in female mice, specifically three liver carcinomas were observed in high dose females with none observed in the control or low dose group.

The increased incidence of hepatocellular carcinoma were statistically significant by trend.

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DR. RICKER: We are now moving on to other relevant data. Data on the pharmacokinetics and metabolism of diaminotoluenes come mainly from animal study and a few studies in humans. The principal isomers studied were the 2,4 to 2,5 and 2,6-isomer and studies used multiple routes and multiple species.

To summarize the overall results, isomers studied have similar kinetics and metabolism across isomer and across species. Absorption is generally fast with oral absorption being faster than dermal. Diaminotoluenes are well distributed throughout the body irrespective of the route of administration, and both parent compounds and/or

metabolites have been detected in blood, liver, kidneys, and other tissues.

The main route of excretion is via urine. Fecal excretion occurs, but is generally a minor route of elimination. Excretion follows a first order kinetics and is generally complete within 24 to 48 hours. Both parent compounds and metabolites are excreted, only very small amounts of diaminotoluenes have been measured in expired air.

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DR. RICKER: Diaminotoluenes can be metabolized via acetylation of amino groups, oxidation of the methyl group and ring hydroxylation. N-acetylation is facilitated by n-acetyltransferase enzymes, and leads to mono- and diacetyl compounds, which have been observed in both humans and animals, and which are excreted via urine and feces.

The methyl group can be oxidized resulting in the formation of N-acetylated aminobenzoic acids. And ring hydroxylation is possible and leads to the formation of 3-, 5- or 6-hydroxy metabolites. Mutagenic metabolites can also be formed and will be discussed later.

With this, I'm concluding my part of the presentation, and handing it over Dr. Sun.

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DR. SUN: Thank you, Dr. Ricker. I will now continue on other relevant data starting with the genotoxicity of the diaminotoluenes, or DATs for short. Because of the large volume of data, this is only a highly condensed table. 3,5-DAT is not included as there is no data. The genotoxicity of 2,4-DAT is listed at the bottom for comparison. The three columns represent three categories of genotoxicity.

The first column shows results from bacteria or yeast assays. All the DAT isomers in this table was positive in inducing reverse mutation in bacteria. 2,5 and 2,6-DAT also induced DNA damage in bacteria. In addition, 2,6-DAT induced chromosomal recombination in yeast.

The next column shows results from in vitro mammalian cell assays, 2,3 and 3,4-DAT were not tested. 2,5 and 2,6-DAT were both positive in assays detecting DNA and chromosome damage, and 2,6-DAT also induced DNA mutation.

The third column summarizes results from in vivo assays. DNA damage was observed for 2,5 and 2,6-DAT. DNA synthesis inhibition was detected for 2,5 and 3,4-DAT. This was reported as a genotoxicity endpoint, because the authors stated the inhibition of DNA synthesis could be due to suppressed DNA template activity, indicating

1 DAT-induced DNA binding or DNA adducts. For more detailed
2 information on each isomer, please refer to the hazard
3 identification document.
4 --o0o--
5 DR. SUN: As Dr. Ricker has mentioned briefly,
6 the metabolism of three DATs has been studied, namely 2,4,
7 2,5, and 2,6-isomers.
8 The following metabolites have been shown to be
9 mutagenic in salmonella: They are the to 2,4-DAT
10 metabolite, 4-acetylamino-2-aminotoluene, and the 2,6-DAT
11 metabolites, 5-hydroxy-2-acetylamino-6-aminotoluene, and
12 2,6-diacetylaminotoluene.
13 --o0o--
14 DR. SUN: This slide summarized the results from
15 in vitro cell transformation studies. For comparison
16 purposes, let's take a look at the last row in the table
17 first. 2,4-DAT was positive in 11 out of 12 studies in
18 Syrian hamster embryo, or SHE, cells. It was also
19 positive in the initiation in mouse Bhas 42 cells. Bhas
20 42 cells are generated from mouse BALB 3T3 fibroblast
21 through the transfection of the v-Ha-ras oncogene. Using
22 two different seeding densities and treatment regimens,
23 the Bhas 42 cell transformation assay has the ability to
24 differentiate tumor initiators from tumor promoters. Now
25 back to the results for the isomers under your

consideration today.

2,3-DAT was considered a positive promoter in the Bhas 42 cell assay. Both 2,5 and 3,4-DAT were weakly positive in one assay in SHE cells and positive in the promotion assay in Bhas 42 cells.

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DR. SUN: 2,6-DAT was weakly positive in one and negative in five studies in SHE cells and negative in the Bhas 42 cells. 3,5-DAT was not tested for cell transformation.

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DR. SUN: 2,4 and 2,6-DAT were studied for their effects on cell proliferation. In an in vivo assay in Fischer rats, the authors treated male animals with 2,4-DAT or 2,6-DAT dihydrochloride by oral gavage for 9 days and examined hepatocyte proliferation. The 2,4-isomer stimulated a dose dependent proliferation, while the 2,6 had no effect.

In an in vitro assay, the authors treated human lung fibroblasts with 2,4 or 2,6-DAT at different doses, two different cell densities and two treatment lengths. 2,4-DAT induced cytotoxicity at multiple doses and at both 24 and 48 hours, while the 2,6-isomer stimulated cell growth at low cell density in highest dosage at 48 hours.

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DR. SUN: OEHHA also identified studies on gene and protein expression changes in cells and animals after DAT treatment, and we discuss them in the document. For today's presentation, I'm going to talk about the effect on apoptosis and cell cycle related genes on this side and effects on cytochrome 1A1 and cytochrome 1A2 on the next slide.

In an in vivo study in rat liver, the authors treated male Fischer rats with daily oral doses 10 milligram per kilogram per day 2,4 or 2,6-DAT for 28 days, and analyzed gene expression with oligo microarrays. Generally speaking, the effects of 2,4-DAT was greater than 2,6. Some of the examples are shown here. Both cyclin G1 and p21 were up-regulated.

For the apoptosis related genes, both Bax and Wig1 were increased by 2,4 and 2,6-isomers. 2,4-DAT induce expression of complement component 7 or C7 and Fas receptor, while 2,6-isomer had a slightly inhibitor effect on these two pro-apoptotic genes.

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DR. SUN: Now, moving on to studies on CYP1A and CYP1A2 expression. In one in vitro assay in the mouse embryo fibroblast cell line, 2,6 -- 2,4-DAT induced a more than six-fold increase of CYP1A1 MRA at 24 hours. CYP1A1 carries out n-hydroxylation of aromatic amines.

N-hydroxylation is known as the first step of aromatic amine induced genotoxicity and carcinogenicity.

In one in vivo study, Cheung et al., 1996 gave intraperitoneal injections of 2,3, 2,4, 2,5 or 2,6-DAT in Wistar rats and examined the protein expression of CYP1A1 and CYP1A2 in the hepatic microsomal protein extract by western blot. 2,3-DAT induced a dose dependent increase of CYP1A1 and CYP1A2 expression.

2,4-DAT also increased CYP1A expression, but the response was not as strong. The antibody they used could not differentiate CYP1A1 and 1A2 in the case for 2,4-DAT.

Cheung at al., also found both 2,3-DAT and 2,4-DAT bind to aryl hydrocarbon receptor, or AHR, which transcriptionally regulates the expression of CYP1A1 and CYP1A2. 2,5 and 2,6-DAT had no effect on CYP1A protein expression.

--o0o--

DR. SUN: More data were identified from the U.S. EPA ToxCast database. ToxCast is a chemical screening program which uses high throughput in vitro assays to detect chemical activity in primary cell culture, cell lines, or isolated proteins. OEHHA identified ToxCast data on three DAT isomers, which are 2,3, 2,4 and 3,4 isomers. The other isomers, 2,5, 2,6 and 3,5-DAT were not tested.

Detailed information on the active assays can be found in Appendix C of the document. Here are some highlights of the ToxCast data. For 2,3, 2,4, and 3,4-DATs, the following were observed:

Upregulation of transcription activity of AHR in human liver cancer cell line HepG2 cells. This was repeated in two assays. Upregulation of the protein expression thrombomodulin, an AHR regulated gene. Also, inhibition of the protein -- inhibition of the proliferation of human skin fibroblasts. In addition, 2,3 and 2,4-DAT inhibited the enzyme activity of a human protein tyrosine phosphatase, which is a tumor suppressor. And 2,4 and 3,4-DAT inhibited enzyme activity of brain type creatine kinase which has been shown to be downregulated in human cancer tissues.

There are also 26 assays that the two ortho-DATs, namely 2,3 and 3,4-isomers were both active in including nine assays showing upregulation of transcription factor activities. Upregulation of protein expression of IL-8 and thrombomodulin both of which are associated with cancer.

Counterintuitively, there is also evidence on downregulation of the expression of cancer associated proteins. The examples here are all associated with cancer progression and metastasis. Also, there is

evidence on inhibition of cell growth or proliferation in two assays.

--o0o--

DR. SUN: Now, we're going to talk about structure activity comparisons. Here is a chemical structure of 2,4-DAT and the other five DATs. DATs were compared among the group and with four structurally related chemicals. They are p-Cresidine, 2,6-xylidine, o-toluidine, o-phenylenediamine. These four chemicals were selected because they were also monocyclic aromatic amines and are activated like DATs. For example 2,6-xylidine and o-toluidine for go through n-hydroxylation to form reactive intermediates. And all four chemicals are Prop 65 carcinogens.

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DR. SUN: This summary table compares the animal tumor sites of the DATs and the comparison chemicals. The endpoints that are being compared are tumor size in rats and in mice. The table also shows the status under Proposition 65. NT means not tested. A letter M or F in parentheses indicate the tumor was only found in male or female animals at a particular site respectively.

There are some similarities of tumor sites. For example, 2,4-DAT, 2,6-DAT, p-Cresidine and 2,6-xylidine all induced liver tumors in rats.

2,4-DAT, 2,5-DAT, p-Cresidine, o-toluidine, o-phenylenediamine all induce liver tumors in mice. Other common tumor sites that were induced by more than one chemical include bladder, lung, nasal, subcutaneous, and vascular tumors.

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DR. SUN: OEHHA also used quantitative structure activity relationship, or QSAR models to predict the carcinogenicity of DATs. QSAR predictions were made for 2,3, 2,5, 2,6, 3,4, and 3,5-DAT, but not for 2,4-isomer, because the 2,4-isomer is individually listed as a carcinogen and is included in the training sets of all the QSAR models.

Only predictions with good reliability were included in the summary table. Those that were against experimental data, or with low confidence levels, were not included. The CAESAR carcinogenicity model predicted 2,3, 2,5, 2,6, and 3,4-DAT to be carcinogens and 3,5-DAT to be a non-carcinogen.

The Lazar model predicted all five DATs to be carcinogens. The OECD toolbox predicted 2,3 and 3,4-DAT to be non-carcinogens in mice, and 2,5, 2,6, and 3,5-DATs to be carcinogens in rats. The NAs here mean results were not available for these isomers.

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DR. SUN: This table summarized the comparison of genotoxicity and cell transformation studies among the DATs and with the comparison chemicals. The first three columns list the three categories of genotoxicity. They are mutagenicity, chromosomal effects and DNA damage and other effects. And the last column here shows the effects on in vitro cell transformation.

As you can see, similar to the 2,4-isomer and the four comparison chemicals, all DATs, except for the 3,5-isomer showed positive genotoxicity in at least one category. And they were all positive in the cell transformation assays.

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DR. SUN: This table shows the QSAR predictions of mutagenicity. All three from the VEGA platform and the Lazar model predicted five DATs to be mutagenic. The predictions by the OECD toolbox are not presented because some were in contrast with experimental data.

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DR. SUN: Now, let's take a look at the possible mechanisms for the carcinogenicity. The most probable mechanism is genotoxicity. We have shown you the summary of the evidence. More information on the formation of DNA reactive intermediates will follow on the next slide. Next one is the receptor-mediated mechanisms, such as the

AHR mediated mechanism. The ToxCast database offers some data on other receptors, such as estrogen receptor, PPARs, and androgen receptor, but those were rather preliminary and have not been verified by other studies.

Therefore, only the AHR-mediated mechanism will . be discussed in more detail today.

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DR. SUN: Here we provide the possible scheme on how DATs induce genotoxicity through formation of reactive intermediates. The figure is for 2,6-xylidine, but the principle applies to DATs as they are both monocyclic primary aromatic amines. It is possible for all DATs to form aryl nitrenium ion upon n-hydroxylation by CYP1A1 or CYP1A2. Nitrenium ion can form covalent DNA adducts and eventually mutations. The n-hydroxylation of DATs by rat liver microsomes has been measured.

2,5-DAT show the highest activity, followed by 2,4-isomer. 2,3 and 2,6-DAT showed lower but measurable activity. Another type of intermediate is the quinone imine structure. This is possible for all DATs, except for the 2,5-isomer in which the para-position for one amino group is occupied by the other.

Last, but not least, reactive oxygen species could be formed from the quinone imine or from protein adducts.

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DR. SUN: Based on the data that are currently available, the most probable receptor mediated mechanism is the AHR-mediated pathway, which has cross talk with a genotoxicity mechanism. And here is the proposed scheme.

DAT binds to the AHR in the cytoplasm, which then gets activated and translocates into the cell nucleus activating the transcription of AHR-regulated genes, CYP1A1 and CYP1A2, which share a bidirectional promoter. The two enzymes that metabolically activate a DAT to reactive intermediates and then lead to genotoxicity.

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DR. SUN: This slide recaptures the evidence that supports the AHR-mediated mechanism. The evidence for the 2,4-isomer is listed in the last row for comparison.

First, 2,3-DAT has been shown to physically bind to AHR. Also, 2,3 and 3,4-DAT upregulate AHR transcription activity based on two reporter assays from ToxCast. Consistent with the reporter assays, there is evidence on upregulation of AHR-regulated genes, either at the mRNA or protein level.

Another piece of supporting data come from the mutagenicity. It has been shown that the mutagenicity of 2,5, 2,6, and 3,4-DAT is greatly increased when the S9 is extracted from beta-Naphthoflavone or beta-NF induced

rats. Beta-NF is a strong AHR agonist and induces the expression of CYP1A1. This shows the AHR activity strongly correlates with the DAT genotoxicity.

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DR. SUN: Okay. Now, that we've shown you the evidence regarding the carcinogenicity of the five DAT isomers, here is a final summary to refresh our memory. The first column shows the tumors in animal bioassays with 2,5-DAT, testicular tumors in male rats and lung tumors in female mice were observed. With 2,6-DAT, liver tumors in male rats and female mice and pancreatic tumors in male rats were observed. 2,3, 3,4, and 3,5-DAT were not tested in animals.

The next three columns are the genotoxicity endpoints. All DATs, except for 3,5-DAT were positive in at least one category of genotoxicity. They were also positive in the in vitro cell transformation assays. There is evidence of AHR activation for the 2,3 and 3,4-isomers. And all five isomers have been predicted to be carcinogens by at least two QSAR models.

With this slide, we conclude our presentation today on diaminotoluenes.

Thank you very much.

CHAIRPERSON MACK: Thank you, Dr. Sun.

Does anybody on the panel have any questions for

either Dr. Ricker or Dr. Sun?

I have one for Dr. Ricker. Do we know anything about the lung -- the natural history of the tumor of the interstitial cell tumors in testis?

DR. RICKER: You mean in terms of numbers that are known?

CHAIRPERSON MACK: Do they kill the mice?

DR. RICKER: Pardon me?

CHAIRPERSON MACK: Do they kill the mice?

DR. SANDY: So these tumors were observed in the rats in the set of studies. And we have discussed in the pathology section of the HID that, in general, these tumors are -- the diagnosis -- differential diagnosis is based on size of the lesion. So as a lesion gets bigger, it's classified as -- from a hyperplasia to an adenoma to a carcinoma. And there are -- they are known to progress, but I don't -- I don't know that they kill the animal. It looks like David has something

COMMITTEE MEMBER EASTMOND: Let me make a comment. Apparently, the survival in the treated was greater than in the controls. So it's unlikely that they were -- caused death, because almost all of them had a tumor.

COMMITTEE MEMBER DAIRKEE: I have a question

CHAIRPERSON MACK: Dr. Dairkee.

COMMITTEE MEMBER DAIRKEE: So is there some understanding of whether the mixed DATs have a synergistic effect, whether by comparison with the 2,4? How does -- is there any idea of what the mixed carcinogenicity is, whether it's with elevated or at the same level? Is there any understanding of that?

DR. SUN: We did not identify any studies that used a mixture of 2,4 and 2,6 mixtures in animal bioassays.

DR. SANDY: I can say that there were studies of complex mixtures containing one or more DATs, but they also -- and those were reviewed in that document, but there -- they were also -- the mixtures included other carcinogens besides diaminotoluenes.

COMMITTEE MEMBER DAIRKEE: But my question is whether that mixture is more potent than individual ones. And the most potent is 2,4, as the data shows. So that kind of comparison doesn't exist, I gather?

DR. RICKER: Yeah. I don't think we found any evidence.

CHAIRPERSON MACK: There being no other questions, then we'll go to the outside people.

I guess the first will be Yeroushalmi and Yeroushalmi.

Yes

DR. SANDY: Did you want to turn to the leads or do you want -- it's up to you, Dr. Mack.

CHAIRPERSON MACK: I couldn't hear you.

Okay. We're going to go to the members of the Committee first then. So the first is Peggy Reynolds. Can you tell us anything about the epidemiology information on this?

COMMITTEE MEMBER REYNOLDS: So I could -- so the epidemiologist usually doesn't have too much to say for these -- these sessions. And I could quote from the executive summary that's prepared by OEHHA that no studies in humans were identified in the literature specifically designed to investigate the risk of cancer associated with exposure to one or more of the DAT isomers. And I suppose I could stop there, but I think it might be worth just a very quick review of the rather extensive human health evidence that was provided from the IARC publications and was provided to the Committee in this document.

So given as I understand that the various isomers of diaminotoluene are structurally similar, and based on what I just heard and what we read here, have some similar activities and some similar evidence of mechanistic influences on carcinogenesis. And given that there is not a single isomer, but some mixture of the isomers that apparently are available in consumer products, which is

where we go if we're going to take a look at human health evidence. And there's been a fairly extensive literature looking particularly at implicating 2,4-DAT, which there's no question here I believe in terms of its carcinogenicity, and taking a look at how it has been historically a very prominent ingredient in hair dyes, particularly dark hair dyes, there's a fair amount of epidemiologic literature that has tried to zero in on this, and various threads of evidence that may be relevant in terms of thinking about the issue of specific isomers versus the mixture issue that we've been discussing.

So, as is usually the case, the epidemiologic literature doesn't directly address risk relationships for specific isomers, nor really even DAT mixed. The epi literature, as summarized for us here, has tended to focus on several different types of studies trying to come at this from a variety of perspectives.

Occupational studies, a logical place to start, in terms of industrial workers handling polyurethane foam; cosmetologists with potential for exposure to hair dyes; and occupation as it's been reported in cohort or registry based studies.

So there also have been quite a number of cancer specific case control studies in which there's been one or more questions about use of hair dyes and its influence on

case status. And then we were also presented with some very interesting evidence from the breast implant studies.

So taking a look at the occupational cohort and the registry studies, there is -- there have been really three studies looking at polyurethane foam workers, one in the UK, one in Sweden, and one in the United States. In each case, generally speaking, although the UK study noted some elevated incidence in mortality for women, for lung and pancreatic cancer, these were all based on very small numbers, and by and large the workforce was too young to really have had sufficient power to take a look at cancer outcomes.

The cosmetologist studies are real quite interesting. There's been a very long-term interest in taking a look at the kinds of exposures that cosmetologists have. They've been nicely summarized for us in the document, and from both the two cited IARC monographs, 57 and 99, which was published in 2010, the most recent. And so dating back really to the late 1950s, there have been numerous studies looking at the cancer experience in this occupational segment.

Cosmetologists generally, at least in California, refers to hair dressers and manicurists. Historically, this workforce has been dominated really by hair dressers. It's only been more recently that the manicurist segment

has increased in terms of the workforce.

So if, in fact, cancer risk is increased with exposure to DAT, and in particular 2,4-DAT, which is listed as a prominent ingredient in hair dyes, particularly dark hair dyes, it might make sense that people who work with this daily, routinely would have a higher risk of cancer than people who are occasional users.

The problem is that people in these professions are exposed to a host of chemicals, including several, like formaldehyde, that are established carcinogens, and that are also present in a number of hair care products.

So the findings really from these studies of cancer incidence or morality from hair dressers or barbers have been very mixed. There have been various reports suggesting workforce members have elevated cancers of the bladder, lung, ovaries, endometrium, and of particular interests, non-hodgkin's lymphomas. Although a number of the studies do cite incidence or mortality from smoking related cancers few or any -- almost none of them have information on smoking, which make it very difficult to interpret the degree to which occupational exposure might have played a role.

And most of the findings for specific cancers, again even in large cohort studies, are based on very

small numbers, so are really underpowered to address the issue.

In the case control studies -- there have been -- case control studies have largely focused on some of the cancers that were highlighted from the earlier occupational studies. The -- well, first of all, cohort studies -- there have really been only a couple of cohort studies. The Nurses Health Study asked questions about hair dye use, and so did the cancer prevention study too. And in both cases, the evidence has been fairly equivocal, there aren't that many individuals who really had the sites of interest, and so they're largely uninterpretable.

The case control studies really dominate the literature. And probably the most informative is National Cancer Institute's U.S. National Bladder Cancer Study, which was published back in 1983. This was a very large study with almost 3,000 cases of bladder cancer and nearly 6,000 controls. They adjusted for smoking. They did a very careful assessment, and found no association would ever use hair dyes, or for length of use.

But for both men and women who reported using black hair dyes, there were significantly elevated odds ratios, again implicating the prominence of at least 2,4-DAT in the formulations for dark hair dyes.

A number of breast cancer studies have been

mostly null. Although, there's been some suggestion of higher risks for those using oxidative or permanent hair dyes. And some of the more interesting have been those studies of the lymphomas and the leukemias, but again, quite mixed and numbers tend to be small.

I thought I just I might mention a little local color, since California has actually been the site of some of the studies, which have book marked this literature. One of the earliest studies really was a 1977 Los Angeles study, authored by Herman Menck, who took a look at the occupational information in the cancer registry collected by the Los Angeles Cancer Surveillance program, and found beauticians to have two-fold elevated proportional incidence ratios and standardized incidence ratios for lung cancer, but, of course, in this case, did not have any information on smoking, again, making it difficult to interpret what that might mean.

One of the more interesting case control studies of bladder cancer comes from Los Angeles and was a study -- series of studies published by Gago-Dominguez starting in 2001, in which she essentially found no association or ever working as a barber or hairdresser, but a significantly increased risk among those with over 10 years of duration of employment. And one of the more interesting findings from her series of studies was that

there did appear to be a considerably elevated risk among those who use permanent hair dyes and were NAT2, N-acetyltransferase 2, slow acetylators, suggesting people who are less able to necessarily clear the chemical in their system.

And one of the most recent studies, which actually was not cited in the monograph, because it was published the same year as the monograph, is our own study -- a study from my own research group of looking at a roster of over 300,000 licensed cosmetologists in California, and we did not find any elevated incidence of cancer in that cohort, with the exception of significantly elevated proportional incidence ratio for thyroid cancer among hair dressers, a completely different cancer than we've been talking about in terms of all of this.

So the breast implant studies, which were cited a little more extensively in this document, were interesting because they're a little more direct in terms of the chemical exposure, since they -- this breast -- at least the Canadian breast implant study took a look at risks -- cancer risks associated with women with and without implants with the polyurethane coating, which was shown to degrade to 4-DAT, which has already been implicated as a carcinogen. But these studies really don't inform the assessment of other isomers.

1 So generally, I would say that the studies of the
2 polyurethane workers have been too small to really draw
3 any conclusions. The studies of cosmetologists have been
4 quite mixed, although implicating again 2,4-DAT, which is
5 really not on the table for discussion.
6 The studies of personal use of hair dyes have
7 been largely mixed. Although, there's been some
8 interesting evidence from individual studies. And the
9 breast implant studies, again interesting, suggesting
10 short-term cancer risks, but not really informative in
11 terms of the range of all six isomers nor mixed -- the
12 mixed.
13 So I note that the Personal Care Products
14 Commission in public comment has raised an issue specific
15 to the isomer 2,5-DAT, submitting evidence that it
16 separately does not appear to meet the criteria for
17 classification as a carcinogen. And asserting that it now
18 is the only isomer present in hair dye products, no longer
19 2,4-DAT. And so I would be quite interested in the
20 comments of my colleagues in terms of their agreement with
21 that.
22 So, again, the epi studies really can't provide
23 information on specific chemicals of interest, nor in
24 particular whether only 2,4-DAT or other specific isomers
25 or DAT mixed should be classified as causing cancer.

They're all the normal limitations of epi studies, sample size, power, lack of covariates, specificity of exposures, and perhaps importantly timing of exposure, since there have been changes in formulation over time for a lot of these products.

And many of the studies that have really implicated DAT are older studies, which probably are, if you could implicate one of the isomers, would probably be 2,4-DAT. So I think what we need to do is consider this in conjunction with the tox evidence, which I'd hoped would come first, so I'd hear what my colleague had to say.

(Laughter.)

COMMITTEE MEMBER REYNOLDS: And I really would like to hear some discussion about the degree to which the isomer 2,4-DAT is present along with other isomers in consumer products. That, to me, is important, in terms of assessing the probability of human health risk. And, so by itself, as nicely stated, already by OEHHA, the Epi literature by itself provides very little information specific to the question on the table versus specific isomers or mixed, but together provides some threads of evidence that are consistent with some of what we've heard about activity for these various isomers.

With that, I'll stop. So that's my long way of

saying, no, there's really nothing in this.

CHAIRPERSON MACK: Thank you, Dr. Reynolds for a very compulsively exhaustive review.

(Laughter.)

CHAIRPERSON MACK: Now, we turn to Dr. Eastmond.

COMMITTEE MEMBER EASTMOND: Thank you. And basically I thank the OEHHA group for putting together the document, and making certainly an argument for listing.

I have a really very different sort of interpretation or read of the data, and I'll go through this. It seems to me since we know what the -- you know, there's five isomers, and 2,4-diaminotoluene clearly should be listed. It already is listed, and it's a very strong carcinogenic, mutagenic, the like.

But let me go through the individual isomers and kind of my take on them.

So basically, you have to 2,3-diaminotoluene, the 3,4 and the 3,5-diaminotoluenes. And there really have been no cancer studies conducted on these. Okay. So it's -- from my point of view, you'd have to have really overwhelming evidence to try and -- from other evidence to try and cull them to list them. And I just don't think that evidence exists.

In most cases, there's basically negative or weekly genotoxic, at least in my reading this. When you

see something that inhibits DNA synthesis, that doesn't automatically mean genotoxic to me, or mutagenic. So those -- I just think there's just not enough evidence to even, you know, consider listing them, but let me go to the other two.

So there's 2,4-diaminotoluene sulfate. And I'll go through this kind of point by point. So this was -- there was a study done in -- by the National Cancer Institute in basically rats and mice. And as indicated, there is a so -- there was an increase in testicular interstitial cell tumors, which have an extremely high incidence in the controls.

We're talking -- actually, there's an errata that apparently was produced that the spontaneous incidence tends to be 80 to 90 percent in the controls. And so it went up to like 95 to 98 in the treated. That is not particularly impressive. And, in fact, since the document, the errata corrects only the high doses significantly increased based on the Fisher exact test.

But one of the things I referred to is if you look at the NCI who evaluated this, they concluded that they did not think that this -- the increase in tumors was attributable to the compound, because of the high spontaneous incidence of these neoplasms in male Fischer 344 rats, because it was both high and variable. So they

didn't think this was a significant increase and I would agree with them on that.

There was no increase in Fischer 344 -- the female Fischer 344 rats, no increase in cancers. In the female B6C3F1 mice, there was an increase in alveolar, bronchiolar, adenomas, and then combined adenomas and carcinomas. And this was seen by pairwise testing.

But there was kind of -- and this is really looking at sort of the high dose and the low dose. There was a very peculiar study as kind of was mentioned is they have -- the low dose had different controls than the high dose. And so if you bring in -- if you look at this, there's a lot of variability between the controls between the low dose and the high dose controls are highly variable.

So whereas there was a significant increase with the high dose compared to its control, if you compare it with the other control, it's not significant. So you have this real variable sorts of thing that's seen. Very unusual.

And as I mentioned, they were housed in different rooms, and they came in at different times. So it was a strange sort of study -- experimental design. And again, in this case, the NCI concluded that there was not sufficient evidence to conclusively demonstrate that

2,5-diaminotoluene sulfate was carcinogenic in either Fischer 344 rats or mice. So basically, the NCI didn't think the evidence was sufficient to list -- to call these carcinogenic, and I would agree with that.

As far as the genotoxicity, the 2,5 is genotoxic in vitro in sort of mammalian cell assays, but usually under conditions of very high -- what appear to be very high doses or cytotoxicity. And we tend to discount that. It was very weakly mutagenic in the Ames test, and less hydrogen peroxide was added. And then it actually became much more mutagenic.

But the interesting thing for me is it was tested in 11 different reports for in vivo genotoxicity -- well, 12 different reports. It was negative in 11 of the 12. And in the 12th one, it was positive in one tissue. I'm not sure how many different tissues that were tested. It was in stomach and how many time points, so -- and it was positive for the comet assay for causing -- so basically DNA strand breaks in the stomach, and that would be at the site of exposure or you could easily have irritation or toxicity. And the comet assay is very prone to problems under those circumstances.

So that's kind of a long rundown on this.

My take on this, and there's -- some of the people will probably comment on this. But, for me,

it's -- I don't know if you want my bottom line on how I would look at it? For me, this hasn't been clearly shown by scientifically valid testing, according to generally accepted principles to cause cancer. It's suggestive, but it's not sort of clearly shown.

Let me to the last one, which is the 2,6-diaminotoluene dihydrochloride. And it's got some of the similar sorts of issues with it. There was a significant trend seen for hepatocellular adenomas and adenomas and carcinomas combined in the male Fischer 344 rats. So these were trend tests and the P values are like 0.03, so they're kind of weakly significant.

However, usually what you want is a tread, and then one of the doses has to be significantly -- statistically significant by itself, and none of these were significant. The individual doses were not. They did comment that these are rare tumors in Fischer 344 rats. So that's one.

And then the other tumor type in the male 344 rats were islet cell adenoma of the pancreas, in which there was a trend -- a significant trend. However, again, when you compare the control in the high dose, it was not statistically significant in a pairwise comparison.

Historical incidence of this particular -- oh, sorry. The spontaneous incidence -- these are commented

as benign tumors. And the spontaneous incidence ranges from 0 to 10 percent. So this -- the high dose falls within the spontaneous, but the average is about four percent. So it is elevated, but it's not significant in some ways.

The other tumor type is in the female mouse, the B6C3F1 mouse. There was a significant trend for hepatocellular carcinomas that they pointed out. This was not seen for the adenomas or the adenomas and carcinomas combined. So -- but the pairwise -- again, on the pairwise comparison, it was not significant, and the increase fell within sort of -- a historical incidence averages eight percent, and this was actually the low -- the high dose was actually below the average for historical control incidence.

So again, the National Cancer Institute in their evaluation of this study concluded that the 2,6-diaminotoluene hydroxide was not carcinogenic in male Fischer 344 rats, because both the incidences of hepatocellular tumors and pancreatic islet cell adenomas were not significantly increased from the controls based on the fisher exact pairwise comparison.

Similarly, the NCI determined that this compound was not carcinogenic in the female mice based on a non-significant increase in tumor incidence between

1 treatment groups and controls by pairwise comparison.
2 They considered the high dose incidence was well -- well,
3 I guess my conclusions is the high dose incidence was well
4 within the historical control range.
5 Again, this is another one where you've got
6 mutagenic effects seen in vitro, but the in vivo studies
7 are largely negative. Actually, 25 in vivo studies kind
8 of crudely counting it. It was negative in 20 of the 25.
9 And those that were positive tended to be flagged for sort
10 of having methodological problems, either quite high doses
11 or inconsistent sort of results.
12 I might point out it was tested in six transgenic
13 mouse or rat assays for mutations. It was negative in all
14 six of them. And that's -- if you have sort of positive
15 Ames test, that's a -- you look in vivo, and these are
16 comparable tests in vivo, and it was negative.
17 So again, my sort of initial conclusion, before
18 hearing the evidence, is that I don't believe this has
19 also been clearly shown through scientifically valid
20 testing, according to generally accepted principles to
21 cause cancer. Again, suggestive, but not clear evidence.
22 CHAIRPERSON MACK: Thank you, David.
23 Let's now go through the other members of the
24 Committee to see if they have any comments or suggestions.
25 Start with Dr. Zhang.

COMMITTEE MEMBER ZHANG: I don't have anymore comments. I think the two leading discussants did a good job, but I do have one question for David. For the in vivo study, you mentioned the three positive and mostly negative. What do you think of the quality of the three positive studies, you know, the results?

I think one way we look is more studies show positive -- a negative and fewer studies show the positive studies. But what is your sense about the quality of the studies, basically, that's my question?

COMMITTEE MEMBER EASTMOND: I assume you're talking about the 2,6-diaminotoluene?

COMMITTEE MEMBER ZHANG: Um-hmm.

COMMITTEE MEMBER EASTMOND: Okay. I mean, I will admit I didn't go back and look at the -- you know, these in specific, but they were kind of flagged with the idea that, as I recall, you know, there's nothing -- these aren't -- again, there's sort of inconsistencies where you see things at a low dose, but not at a higher dose. You know, so then what do you -- you know, that's the one that they looked at their dosing regimen.

Now, there are differences in length of time, so it may be that it's a chronic sorts of thing. I mean, I didn't go through it in the great detail, because for me, the cancer data itself is not, you know, sufficient. The

genetox data is largely negative. There are some positives, so -- but it's hard to have that push for me. You have enough evidence or weight to shift the weight of evidence, but I didn't go through in great detail.

That's happy to do so, if you'd like me to.

COMMITTEE MEMBER ZHANG: Thank you, David. There is my long-term colleague. I always rely on him. He is very careful reading, so that's why I was asking about his detailed comments.

CHAIRPERSON MACK: Dr. Dairkee.

COMMITTEE MEMBER DAIRKEE: Yeah, from a cell biologist's perspective, I would say the genotoxicity is striking to me, because several tests have been done. And in vitro, in vivo, and it seems there is genotoxicity. And furthermore, it is supported by the inhibition of cell proliferation. That's what happens when there is genotoxicity, cells stop to grow, and they repair. Cell cycle is arrested, that's why there's growth inhibition. And I find that to be very supportive of the genotoxicity data.

So, now again, where does cancer come from? It doesn't come from cells that have stopped growing. It comes from cells that grow, so -- but in my lab, we have recently published that there are other genotoxic chemicals that will also promote cell survival. So they

don't really spin the cell off on the cell death path, but instead they promote cell survival.

And so if you're causing genotoxicity, promoting cell survival, then whenever that toxic chemical is not around, those cells take off, start growing again. So it's not a permanent inhibition of cell proliferation. It can be reversed, and coupled with genotoxicity, it can be a time bomb. So it's not a good thing to have cells that know how to grow and have DNA damage or other kinds things.

So I'm concerned about the fact that these agents are -- these chemicals are showing complementary effects in terms of the cell biology.

CHAIRPERSON MACK: Joe.

COMMITTEE MEMBER LANDOLPH: Yeah. I appreciated the epidemiology discussion by Peggy. It was very helpful. I am convinced there are problems here with these compounds, as shown more strongly in the occupational usage of them, than in the usage by individual people, but there are mixtures. They're very conflicting, I agree.

I'm very impressed by the genetox base here that these compounds are all metabolized in a similar way. They all bind to DNA. So that's very straightforward. They're clearly a class. Looking at the table, since we

can't do much with the epidemiology data, we're kind of forced to rely on the tumor data. So I would not consider 2,3, or 3,4, or 3,5 any further, because they just haven't been tested in animals.

So I agree with Dr. Eastmond, we've got an extensive genetox database, but we can't rely on just cell transformation or just the genetox data. They have to actually be tested as far as I'm concerned.

I think relying on QSAR. I'm a little bit of luddite in this instance. I think that can get you into trouble, unless you're forced, you know, by over-honing means to use it. I would rather not do that.

So I would look at the 2,4, which I think is already a settled itself issue, the 2,5 and the 2,6. And the 2,5 and the 2,6 are both extensively genotoxic. The responses vary across assays. But they are positive for mutagenicity and bacteria and chromosomal effects, DNA damage and other effects. In vitro transformation. That assay is a little bit shakier than I would like the SHE cell assay.

There are problems with the animal databases. But in looking at the very extensive summary by OEHHA in the hazard identification document, I think that data is significant, when coupled with the extensive genetox positive database that I would be prone to list the 2,4,

the 2,5, and the 2,6 as carcinogens and stop at that point. And then we have to fight over what you're going to do with mixtures.

CHAIRPERSON MACK: Thanks, Joe.

Jason.

COMMITTEE MEMBER BUSH: I haven't really got much to add. I think the Panel has stated most of that. I'm basing my opinion on the predominance of the genotoxicity data.

I agree with David that the NCI studies -- I've been going back 30 years for those experiments were probably flawed, to some extent, and not very informative in terms of the tumor burden there. So I think the weight of the genotoxicity data is convincing for me.

COMMITTEE MEMBER EASTMOND: Tom, can I --

CHAIRPERSON MACK: Well, I basically am left anxious, because I really think these compounds have not been adequately studied, which means it's really difficult to go for a listing in the absence of solid information, but the genotoxicity data bothers me also.

But let's see what the outside individuals can do to -- yeah, David.

COMMITTEE MEMBER EASTMOND: Yeah, I'll just make a comment. If you look at the genetox data, and really the in vivo is where I think is most important, it's

1 overwhelmingly negative for these compounds. And the
2 where it's positive tends to be in the comet assay, or,
3 you know, so you have one positive in the micronucleus.
4 You've got five or six negatives. You have one common the
5 comet assay, or two. And the comet assay is notoriously
6 prone to problems. And so, for me, this is not a
7 convincing case for, you know, in vivo genotoxicity.
8 And if anyone wants to look at page 82 and 83 in
9 the original document, is what you're looking at, for the
10 in vivo for 2,6-diaminotoluene. But a similar sort thing
11 with the -- basically, with the 2,5. And that one, it's
12 actually a little misleading, because they only showed the
13 one positive result and didn't talk about all the other
14 tissues that were negative or the time points that were
15 negative on the 2,5. So, you know, it's very hard without
16 actually digging back into this to figure out what the
17 data actually shows.
18 CHAIRPERSON MACK: Doesn't bother you that the
19 2,4 is clearly different than the others?
20 COMMITTEE MEMBER EASTMOND: Oh, it's clearly
21 positive. And, you know, from a point of view from sort
22 of a classic metabolism, one of the key questions, if you
23 get the hydroxyl amine, this makes you nervous, because
24 that's sort of classic stepware. You would end up
25 potential get a nitrenium ion and carbonium ion formed.

But, you know, the proof is, it's not -- the evidence isn't there. I mean, it's certainly suggestive, and this is a class is suggestive, but for me it's just sort of the clearly shown issue that drives my thinking on this

CHAIRPERSON MACK: Okay. Now, it's time to go on.

CHIEF COUNSEL MONAHAN-CUMMINGS: Dr. Mack, you might want to ask for the other speaker cards as well, not just the --

CHAIRPERSON MACK: I can't hear you Carol.

CHIEF COUNSEL MONAHAN-CUMMINGS: You want to ask for other speaker cards?

CHAIRPERSON MACK: Oh. Do we have speaker cards?

ACTING DIRECTOR ZEISE: Yes.

CHIEF COUNSEL MONAHAN-CUMMINGS: I guess not.

CHAIRPERSON MACK: No speaker cards. But we do have requests from three groups to give presentations. And so I would now start with Yeroushalmi and Yeroushalmi.

(Thereupon an overhead presentation was presented as follows.)

DR. MELIKYAN: Good morning. I am Gagik Melikyan from California State University, Northridge, Department of Chemistry and biochemistry. And title of my presentation, "Diaminotoluenes (mix): To List or Not to

List".

Diaminotoluenes are isomeric compounds. What they have in commone is the presence of methyl group and two amino groups attached to the periphery of the aromatic hexagon, and they are related to each other, or positional isomers. And they can be divided into certain groups when the amino groups on the water position to each other or they are main methyl position to each other, with 2,5 being the unique since they are in para position to each other.

In fall 2014, the Big Lots has came up with a petition to delist the diaminotoluene, or DAT, mix from the Prop 65 list. And in December 2014, I provided a declaration --

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DR. MELIKYAN: -- in which the evidence -- well, the petition was analyzed and the evidence was presented that this compound should be listed maybe in some modified version that I will show you at the end of my presentation.

I would like to show a couple of arguments which were given in the Big Lots petition. The first argument by the experts, which prepared this petition was the very nature of the document which was used actually for listing, and 1986 document was presented showing that this

is, in fact, a draft and not the final document.

The truth is that defendant expert was using the wrong document, because the listing was based, not on 1986, but 1988 document. And this is clearly stated that it is not a draft, but this is a final document.

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DR. MELIKYAN: The second argument for delisting presented by Big Lots expert was that listing of diaminotoluene mix is unclear and confusing. In fact, the truth is that the number of positional isomers theoretically possible are not 10, as the expert claims, but only 6. And these are the numbers which are presented here. And the final document from 1988 clearly indicates that this assignment as carcinogenic is based on the carcinogenicity of 2,4-diaminotoluene, and assignment is applicable to all isomers of diaminotoluene, meaning to remaining out of six, 5 isomers. So apparently, it's not unclear and confusing, it's properly explained.

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DR. MELIKYAN: And the third argument for delisting is, in the expert's opinion, is the most correct definition of diaminotoluene mix is a formulation of mixed salts of 2,4-diaminotoluene, and at the very least the mixture must contain, in part, a component of 2,4-diaminotoluene. These are the most puzzling comments

I have ever seen in scientific literature, because it clearly indicates that it's applicable to all isomers of diaminotoluene, and there is not nothing about salt, and there is nothing about obligatory presence of that specific 2,4-diaminotoluene.

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DR. MELIKYAN: And this comment is repeated actually in the 20 plus page document three times, applicable to all isomers of diaminotoluene.

Another reason for delisting, it's recently presented, is that DAT isomers are difficult to determine in cosmetic formulation because of the limitations of chromatographic methods. The truth is that by using the nuclear magnetic resonance spectroscopy 2,4, 2,5, and 2,6 are easily determined, and presence is established in several minutes. We have done it repeatedly.

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DR. MELIKYAN: And the last and fifth argument for delisting DAT is the following:

The problem is that there is nothing in State Proposition 65 listing that defines the -- what the composition of this mixture could be. It is impossible to tell, since Prop 65 listing doesn't include the CAS number. The truth is that it's understandable why Prop 65 doesn't include the CAS number, because it is a single

listing that covers several compounds. When it's more than one compound, especially group of compounds are covered, apparently not the multiple lines in the Prop 65 can be allocated to present all CAS numbers.

And these are just 19 examples from the current list of the Prop 65, in which we have a group listing for whole classes of organic compounds. And none of them, as you can see over here, lead compounds, Chromium VI, aminoglycosides, barbiturates, and of course none of the CAS numbers are given for an obvious reason.

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DR. MELIKYAN: Now, in which case is this single listings are justified? Well, from the scientific point of view, we understand the mechanism of carcinogenicity, and we -- when going from one component to the other, we preserve the key functional groups. Then, of course, it's understandable that they -- there is a very good chance that they will have analogous biological activity or which part of the molecule will be not consequential for the biological activity in question.

I'm giving you, as an example, a female hormone, which is shown over here. These are the sites of the enzymatic hydroxylation. And apparently, this is the warhead of the molecule along with this OH which are the contact points. And they are responsible for conversion

of ortho-hydroquinone in particular to carcinogenic ortho-quinones.

But also there is a silent part of the molecule, and it's a non-consequential one. And if we introduce the methyl groups in these positions, we definitely would anticipate that there won't be too much change. There can be quantitative changes in certain parameters, but it won't be a qualitative change.

So coming up with the current but single listings, the way it is done in Prop 65 for at least 19 cases, is completely justified. Reason one, it is scientifically sound. Reason 2, it's economically efficient. We don't have to test each and every structural analog when the outcome is more or less obvious. Reason 3 societal responsible because it decreases the tax burden -- financial tax burden. And reason 4, it saves people lives, because if we look too long -- you know how long it usually takes to do all these toxicological studies of different types, scientific studies. It takes years and years and millions of people are subjected to this compound.

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DR. MELIKYAN: Now, let me just comment on some of the DAT isomers that we are analyzing today. The first molecule is a 2,4-diaminotoluene, which we'll all agree is

a proven human carcinogen and it is listed. If we look at 2,6 and 3,5, and we analyze that this position of amino groups they all belong to the same class of diamines, which are meta-diamine. So the only difference between these three molecules is the presence of methyl group, which is at the top of the hexagon here between $2NH_2$ and here in a meta 2 amino groups. These are the compounds which are currently under consideration.

So by knowing so much about the oncology of these compounds. Enzymatic transformations of different classes of compounds it is obvious that the chemistry of these compounds, type of enzymatic transformation would be analogous, because they belong to the same type of the compounds, which are meta-diamines.

Here, we're showing the triad of molecules for the proving this point. If we have a benzidine, and this is the core structure for this molecule. The 3,3-dimethylbenzidine analogous to benzidine is a listed compound. It is a known carcinogen. Just because of the presence of methyl groups, it didn't stop being a carcinogen. Even 3,3 prime trimethoxy, still since it is the core structure is there, those are the two criterias that are -- criteria that I was showing at the beginning. It's still there. It doesn't surprise me that all these three compounds are listed as human carcinogens, as they

should be

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DR. MELIKYAN: If we look at three other molecules, in particular, those which has the ortho-diamines, we're talking about 2,3, and 3,4, they don't have a direct analogy as 2,4, for meta-diamines, but they have a very powerful carcinogenic compound in the list of Prop 65, which is called ortho-phenylenediamine. And the ortho-phenylenediamine, of course, the diamine groups are in ortho position to each other. Here, the whole difference that we have again just the presence of one methyl group in different positions on the periphery of the aromatic ring.

How we can claim that this compound is carcinogenic and this compound most probable with be benign to the general public.

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DR. MELIKYAN: The reason why these meta compounds are especially dangerous, because we know the whole story with ortho-quinones. Ortho-quinones are proven carcinogenic compounds. This is the structure of the female hormone. This is a 2-hydroxy and 4-hydroxy. They're enzymatic oxidations. And these are the further transformations of the ortho-hydroquinone 3,4, which is formed over here. It is known that it oxidizes to

ortho-quinones. It is known that ortho-quinones are carcinogenic compounds. It is known the DNA basis can add to this position at the top of the hexagon and to form the adducts.

On top of it, the 40 structures has been already isolated when the nucleophilic biological entities are added to the quinone compounds. And no one is arguing about the fact that the female hormones is a carcinogenic compound and it is a listed compound in Prop 65.

And these are absolutely analogous compounds. We should expect that these compounds will be oxidized. There is plenty of evidence for different type of the dehydrogenation reactions forming ortho-diamines. Ortho-diamines. And these ortho-diamines, analogous to the ortho-quinones, should be able to react with the DNA basis also with nucleophilic amino acids, like cysteine and arginine de-capacitating or incapacitating the key enzymes inside the body.

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DR. MELIKYAN: Overall, aromatic compounds, and unfortunately DAT, all isomers they belong to aromatic compounds, really are not the benign class of compounds. Here is aniline, which is a listed compound. These are just the metal derivatives of aniline with one methyl group -- with three metal groups, and all of them are

listed compounds.

And this is obvious, because the core structure, which causes the aromaticity apparently is not a methyl group. It is just a combination NH_2 group, with the aromatic compound. This is again ortho-phenylenediamine, and it has now two amines. And this one also 2,5-diaminotoluene, which is currently under consideration. It belongs to the same class of, as you can see there, aromatic amines. And overall, aromatic amines are heavily represented in Prop 65 list. These are the 19 examples of aromatic amines of different types, of different topology, of different number of rings, different disposition of the substituents, different nature of substituents.

The only think that they have in common the presence of the NH_2, and the aromatic ring. So the last thing we would like to do to expose the general public to more of these compounds. Truly, the lessons of history are not learned.

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DR. MELIKYAN: And I would like also to show at the end, the compilation of the biological activities, which are known today. This is just a tip of the iceberg. And a very comprehensive review has been provided today but OEHHA specialist.

This is the 2,4-DAT compound over here. And this is its isomeric molecule, which is 2,5. It's shown as mutagenic. It's genotoxic. It's carcinogenic in mice, carcinogenic in rats, testicular and lung tumor. It is true that authors didn't consider this to be sufficient to say that this is proven carcinogen, but it was statistically significant.

On top of it, it is shown to be teratogenic in mice. The fetuses suffered from skeletal malformations, such as fused and distorted thoracic vertebrae associated with absent and fused ribs. So 2,5-DAT not only has all this data behind it, but also it is known teratogenic compound. If you look at the regional paper, and the malformations, which are presented in a very graphic form, it is very convincing. It is a teratogenic compound.

So talking about the 2,6 compounds, 2,6-diaminotoluene, it's mutagenic, genotoxic. Also it's know to produce the DNA damaging free radicals even in larger quantities, that known human carcinogen, which is 4 -- 2,4-derivative. And already I talked about those compounds which are -- which contained amino groups in ortho position.

The fact is that if compounds are mutagenic, then there is a very good chance they would be -- also be carcinogenic, because 90 percent of known carcinogens,

they are also mutagenic, and x-ray and UV radiation, which are, of course, the carcinogen -- have a carcinogenic impact, they also known to be mutagenic.

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DR. MELIKYAN: By knowing so much about this, I would like to suggest that we do the following changes in the Prop 65 listing:

Current listing diaminotoluene mix always clearly explained at the time by OEHHA, what exactly it means; to avoid any kind of ambiguity can be replaced with diaminotoluene isomers and their salts; type of activity, cancer; based on the totality of data, which are presented today and in the literature; and clarification, therefore, the basis document should read diaminotoluene isomers in Prop 65 listing include 2,3, the CAS number; 2,5, the CAS, number; 2,6, CAS number; 3,4, CAS number; and 3,5, CAS number. And should explicitly state that can be found in the consumer products, either individually, or as mixtures of any combinations thereof. And 2,4 should not be there, because already it's a separate listing.

I would also suggest that also there is no listing of 2,5-diaminotoluene as a developmental toxicant. The fact that -- the fact that the teratogenicity is shown, although in limited number of papers, then I think we should introduce this one to keep public safe and to

list it as a developmental toxicant as well.

This concludes my presentation. Thank you.

CHAIRPERSON MACK: Thank you, Mr. Yeroushalmi, is that correct? No.

DR. MELIKYAN: No. My name is Gagik Melikyan. I'm a professor of chemistry at California State Northridge. Yeroushalmi is a law firm.

CHAIRPERSON MACK: They're the law firm. Okay. Just a bunch lawyers. Any way, thank you for your presentation. We certainly know where you stand.

DR. MELIKYAN: Thank you.

CHAIRPERSON MACK: Now, the next one. Big Lots.

MS. BROPHY: I have found that my presentation is going to be a little shorter. Hopefully I can keep it within 15 minutes. And the reason for that is I don't have to talk about the studies dealing with 2,5-diaminotoluene not being scientifically valid because Dr. Eastmond, I believe, covered that statistically. But I do need to explain --

CHIEF COUNSEL MONAHAN-CUMMINGS: Excuse me. Could you identify yourself for the record.

MS. BROPHY: Excuse me. I'm Carol Brophy and I represent Big Lots. And I'm an attorney with Sedgwick, LLP. And I hope that doesn't prejudice me too much in your eyes.

(Thereupon an overhead presentation was presented as follows.)

MS. BROPHY: I would like to say first that I thank you so much for your service. I do recognize that you're here to deal with the science. I also recognize that I am not -- do not have a Ph.D. in science. I'm here to defer absolutely to your opinion. Although, I am going to beg for clarity in all of the listings that you make for cancer going forward. And I would like to rebut a couple of things that my predecessor speaker actually said, but not very many.

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MS. BROPHY: I'd like to start, however, with why I believe you need to hear a little about what happened with the listing of diaminotoluene mixed. I don't say that because I'm asking you to make a legal decision. I am saying that because I think in looking at the history of the listing and understanding what mischief is caused when the listing is not very clear, when the studies on which it is listed are not articulated with sufficient specificity to allow the community that relies on the listing to figure out how to comply with it.

There is no one that wants to expose anyone to a cancer causing agent without a warning, but is sometimes

hard to figure out how and what is actually on the list. And in the case of the Big Lots store, we're a retailer. We don't make this product. We are in litigation because the manufacturer in Europe was never served by the plaintiff because they would have to do so through the Hague Convention, which is too complicated, and they are allowed to sue the retailer for selling it in California without a warning.

When we got the notice, we looked at our product and said, we don't have any diaminotoluene mixed. It's not on the label. We don't -- there is nothing in there. And they said, "Well, yes. Yes, you do". So we went to OEHHA and did something called a Public Records Act request. What that means is our scientists got the documents that were put in the file that's formed the basis of the listing. And based on the listing documents, which I've put up here on the screen behind you, the Public Records Act request, the top document is the notice of the intent to list, which does identify the 1986 document as forming the basis.

CHAIRPERSON MACK: I'm sorry. I'm going to interrupt you because this really doesn't address our responsibility. And I'm -- I'm not -- I'm being sympathetic. I am sympathetic. I think it's really a pain that you have to go through this, but it has nothing

do with our decision. So I'm afraid I have to cut you off, because you're not addressing the science.

MS. BROPHY: May I move to the next point then.

CHAIRPERSON MACK: You may make the next point in a single sentence.

MS. BROPHY: The document that formed the basis of the listing identified the CAS number associated with 2,4-diaminotoluene, and called it -- may I continue with this line? Is this --

CHAIRPERSON MACK: With your sentence. Yes, you may finish the sentence.

(Laughter.)

MS. BROPHY: Please allow me to just show you in the listing document that the definition of 2,4 -- excuse me, the diaminotoluene mixed actually was listed in the document, in both the final and in the initial one, under -- and this -- no entry for diaminotoluene list, both EPA 1986 and 1988, number 77 identifies the CAS number for diamino -- 2,4-diaminotoluene.

CHAIRPERSON MACK: I understand the situation. I understand the problem, but it doesn't bear on what we have to decide, and I really appreciate you're coming, and I sympathize with you, but you can't continue. I'm sorry.

MS. BROPHY: Then if I'm being cutoff, my opportunity to talk to the Panel about the concerns about

the clarity of the listing and requests --

CHAIRPERSON MACK: I respect that.

MS. BROPHY: I am not able to request that whatever you do --

CHAIRPERSON MACK: Will become clear.

MS. BROPHY: -- you will put a CAS number, and I am not able to rebut the comments of the other side who actually said we made false statements in our listing, is that your position, Dr. Mack?

CHAIRPERSON MACK: Our position is that neither of you have anything to do with our problem, and we have to deal with our problem. So I appreciate the confusion that the situation has caused, but we can't help you with that. We just have to make our biologic decision. And I appreciate your coming and thank you very much for your presentation.

MS. BROPHY: Thank you for at least what you did allow me to say. Thank you.

CHAIRPERSON MACK: Okay. First personal care products.

(Thereupon an overhead presentation was presented as follows.)

DR. BJERKE: Thank you, Chairman Mack and thank you, Carcinogen Identification Committee for granting me 15 -- oops.

Thank you, Chairman Mack and Carcinogen Identification Committee for granting me 15 minutes to talk about some of the science behind the diaminotoluenes.

My name is Don Bjerke. I'm a practicing toxicologist. I've been practicing for 22 years. I'm a Diplomate of the American Board of Toxicology. I'm representing Procter and Gamble and the Personal Care Products Council, Hair Coloring Technical Committee.

Next slide, please.

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DR. BJERKE: And, Dr. Reynolds, I would like to address one of the comments that you made. You asked a question about whether 2,4-diaminotoluene is used in hair dyes. It has not been used in hair dyes since the early 1970s, so we can take that off the table.

The statement of the issues that we're here today to talk about are data on 2,5-diaminotoluene. They do not support the listing of this isomer by itself. The listing of diaminotoluene mixed is problematic, because it does not identify which isomers were tested, and it does not identify which isomers are hazardous.

Next slide, please.

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DR. BJERKE: So the reasons why 2,5-diaminotoluene should not be listed as a carcinogen:

The National Cancer Institute and IARC have both concluded the evidence is insufficient to declare 2,5-diamintoluene a carcinogen. The in vivo genetic toxicity data does not show evidence of genotoxicity for 2,5-diaminotoluene. Again, this is specific to 2,5.

2,5-diaminotoluene is not a component of the meta-diaminotoluene or ortho-diaminotoluene mixed isomers. The synthetic pathway is different. And when they -- the starting materials for 2,5-diaminotoluene are different and cannot form 2,4 or 2,6. So when we talk about hair dyes, 2,5 alone is the only isomer that's in that product.

The biological activity of 2,5-diaminotoluene is significantly different than 2,4-diaminotoluene. So not all diaminotoluenes are the same. They may look similar in two dimension on paper, but that doesn't mean they have the same biological activity.

Next slide, please.

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DR. BJERKE: To provide evidence of the difference in 2,4 and 2,5 from a biological standpoint, this is from Table 1 of our written comments, as you can see the only similarity between 2,4 and 2,5 is the in vitro genotoxicity data. So the in vivo genotoxicity data on 2,4 and 2,5 are quite similar. However, the in vivo genotoxicity data is significantly different, as Dr.

Eastmond has made the point as well.

So if we start at the top, if you look at the NCI bioassays, 2,4-diaminotoluene is positive in males, male rats, female rats, and in female mice. Whereas, 2,5-diaminotoluene, according to IARC and according to the NCI, are negative in all of those studies, all of those bioassays. I will argue that the geno -- and I'll talk a little bit more about the specifics of those in a couple slides.

Now, the genotoxicity data in vivo is positive for 2,4-diaminotoluene. I agree with that. But with 2,5-diaminotoluene it's negative. And there some controversy over some of the -- over the comet assay and some of the results. And I'll talk about that in a little bit as well. The IARC classification, as a carcinogen, is yes for 2,4-diaminotoluene, no for 2,5. The NTP report on carcinogens lists 2,4 as a carcinogen, does not list 2,5 as a carcinogen. The EPA tested 2,4-diaminotoluene, and considered it a carcinogen. It did not test 2,5-diaminotoluene.

In fact, use of 2,5-diamintoluene is approved in Europe as a hair dye. The Scientific Committee for Consumer Safety has done a thorough review very recently that has approved the use of 2,5-diaminotoluene as a safe hair dye, active.

1 So the pattern of effects is completely different
2 between these two isomers. So not all of these
3 diaminotoluene isomers are the same when it comes to their
4 biological activity. So we believe that listing should be
5 based specifically on the isomer that was tested and where
6 there is clear evidence of carcinogenicity.
7 Next slide, please.
8 --o0o--
9 DR. BJERKE: Okay. So let's talk a little bit
10 about the testicular tumors in the Fischer 344 rats, as
11 this has caused some controversy. So there is no
12 statistically significant increase in tumors for any of
13 the other tissues, except for the testes in these male
14 Fischer 344 rats. And it's very important to recognize
15 these as male Fischer 344 rats, because the high and
16 variable historical spontaneous tumor rate.
17 So the NCI concluded that these testicular
18 tumors, while statistically significant, were not
19 attributable to compound administration. And, in fact,
20 the NTP historical control data in 1999 looked at Fischer
21 344 rats. And what they did is they took 20 bioassays
22 where they were all fed the same diet as what the
23 two -- the -- as in the study with the 2,5-diaminotoluene.
24 And what they concluded was that spontaneous
25 incidents of testicular tumors range from 74 percent all

the way up to 96 percent. So as you can see, there's a huge background spontaneous incidents that's both very high and highly variable.

Now, unfortunately, these -- this NTP 1999 report did not call out the different labs at which these studies were run. So we can't tell which -- what the actual incidence rate is for Mason Research Institute, which is where 2,5-DAT was tested.

Dr. Eastmond made this note very early on in the proceedings that animals in the treatment groups survived longer, and thus had further time to develop these spontaneous testicular tumors. So this is very important. So if you look at our written comments and you look at the percentage of animals that lived beyond 85 weeks, it's significantly higher in both 2,5-diaminotoluene treatment groups. So these animals had longer time to develop these testicular tumors.

So at the end of the day, the data on 2,5-diaminotoluene does not support clear evidence of carcinogenicity.

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DR. BJERKE: With regards to the lung tumors, so in the mouse study that NCI conducted with 2,5-diaminotoluene sulfate, there were no increased

incidence of lung tumors in male mice. These were only in female mice. Now, NCI and the Clearinghouse on Environmental Carcinogens both recognized the design flaws that limit the interpretation of the lung adenomas and carcinomas observed in the female mice.

So classically, when you have a control group, the controls in the test animals should all be treated the same. They should be from the same supplier, arrive at the same time, be treated concurrently, be housed in the same room, and the only thing that should vary is the dose that's administered. The dose of the test article that's administered.

In this particular study, the controls came from different suppliers. They were housed in different rooms, and they were not started at the same time as the treatment groups. In addition to that, even more concerning, is that these control animals were housed in the same room as active carcinogens that were being tested. So there's a lot of flaws to these studies. So it is difficult to say that these are well accepted studies.

Now, again, with the lung tumors in this particular study, there is quite a variability in the different control groups. As Dr. Eastmond noted, There are two different control groups, one for the low dose

test material, and one for the high dose test material. And the spontaneous incidence of lung tumors vary about four-fold.

And again, as Dr. Eastmond has already indicated, if you took and just happened to run these different controls and match them with the other dose group, the -- so that the 8.9 percent spontaneous incidence of lung tumors in females was with a high dose group, the statistical significance goes away.

So because of these design flaws, the scientific validity of this testing cannot be verified. And NCI concluded that the increased incidence of combined lung tumors does not provide sufficient evidence of a compound related effect.

Next slide, please.

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DR. BJERKE: So now that we've talked about the spontaneous tumors that were seen in the control groups, as well as some of the treatment groups and the bioassays, now let's turn our attention to the in vivo genotoxicity.

So again, we're acknowledging that the in vitro genotoxicity for some of the 2,5 DAT were positive. However, these results did not play out in vivo. So the in vivo genotoxicity takes more weight when you're looking at the overall weight of the evidence than the in vitro

toxicity does.

In fact, for 2,5-diaminotoluene and this is -- this is Table 7 in our written comments, there were two mouse bone marrow micronucleus assays, both negative. There was an OECD guideline GLP compliant on scheduled DNA synthesis study that was conducted. It was negative.

The comet assay was negative in -- for colon, liver, kidney, bladder, lung, brain, and bone marrow. It was only positive in the stomach tissue. Now, as Dr. Eastmond has noted, these studies are quite problematic, and subsequent to running these studies, there have been several publications that have talked about how to improve the quality of these studies to make them more scientifically valid.

So in this particular study, there was one dose group, and only one dose group. There were no historical control data. And there was one slide per tissue per animal that looked at 50 nuclei. So this study is very problematic. It's not a thorough study. And the effects on the stomach, you know, we oftentimes see this in toxicological studies, where we think this is a point of contact irritant response that's leading to the DNA damage in the stomach as an artifact of oral gavage dosing.

In addition, there are mouse spot tests and dominant lethal assays, two each, which were both negative

which look at somatic cell mutation and germ cell mutations as well.

So what we'd like to do is not just look at those -- the outliers, where there are some indications perhaps of a positive finding. We like to look at the overall weight of the evidence, and look at all the studies.

And actually, let me talk about the Greene study as well. So the Greene study was called out in the documents as being a study that -- where they were looking at DNA damage. In fact, this is not a genotoxicity study at all. This is a study that looked at DNA synthesis. So this is considered a geno -- this is considered a cytotoxicity or cytostatic assay. This is not a genotoxicity assay.

In fact, if you want to look at on scheduled DNA synthesis, you go to the guideline study that we have, run under GLP conditions, which was negative. So the Greene study should not be considered at all.

Next slide, please.

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DR. BJERKE: Okay. So now we get into, you know, is 2,5-diaminotoluene a constituent of mixed diaminotoluenes or not?

It is problematic, and with all due respect, Dr.

Mack, if I start going off too much about CAS numbers, feel free to cut me off here.

(Laughter.)

CHAIRPERSON MACK: Do it right away.

(Laughter.)

DR. BJERKE: Do it right away?

(Laughter.)

DR. BJERKE: Okay. I'll summarize very quickly. I've got this and then one more slide.

Right. Okay.

All right. So one point is that the starting materials for 2,5-diamintoluene, this is the hair dye ingredient, is an ortho-aminoazotoluene. It is impossible to have 2,4 in there. It is impossible to have 2,6 in there. There are other methods of synthesizing 2,5-diaminotoluene, but none of them have 2,4 or 2,6 in there, period.

These are different than the mixed diaminotoluenes that industry uses for other purposes. Those tend to be meta or ortho-diaminotoluenes. They do not have 2,5 in there as well.

Okay. Next slide, please.

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DR. BJERKE: So in summary, NCI and IARC have concluded that evidence is insufficient to declare

2,5-diaminotoluene a carcinogen. And we agree with that. 2,5-diaminotoluene has not been clearly shown through scientifically valid testing, according to generally accepted principles, to cause cancer. And we talked about the problems with the lung tumors in female mice and the testicular tumors in male rats. We talked about the difficulties with the comet assay. And we've taken the Greene paper off the table as being completely irrelevant.

In addition, we feel that diaminotoluene mixed should be delisted. First of all, mixed isomers were not tested. Mixed does not identify which isomers are hazardous, and different isomers clearly have different biological activity.

Thank you very much for your time and attention.

CHAIRPERSON MACK: How are you doing? Do you need a break?

THE COURT REPORTER: Yes, that would be great.

CHAIRPERSON MACK: Okay. How long?

THE COURT REPORTER: Ten minutes.

CHAIRPERSON MACK: Five minute -- ten minute break.

(Off record: 12:05 PM)

(Thereupon a recess was taken.)

(On record: 12:16 PM.)

CHAIRPERSON MACK: Okay. I give the floor to

you.

DR. SANDY: Thank you, Dr. Mack. This is Martha Sandy. We wanted to -- since there's been some discussion of the genotoxicity data for the 2,6 in particular, I wanted to highlight that the comet assay where they saw a positive effect in the stomach of the rat, that's actually a paper -- we provided the Committee with all the references cited, with Rothfuss et al. 2010, so that's a fairly recent study. And it was a collaborative study on 15 compounds just to mention that. I encourage you to take a look at that. And then we prepared some information on this one assay, looking at inhibition of DNA synthesis. This was the Greene et al. Paper that was referred to. And that's a very unusual assay. It's not used anymore. And just to familiarize the Committee with that assay, we have a little short presentation by Dr. Sun.

So if you could bring up the first slide.

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DR. SUN: Okay. Because this assay is so unusual, so I will just provide you a very brief introduction and three evaluations of this assay. So Greene et al. 1981 they treated animals, these mice, with DATs via IP injection, three hours later labeled -- injected -- radiolabeled a thymidine analog. And then 30

minutes later they removed the testes and examined the radioactivity in the DNA. So one major concern about this assay is the reduction of testis temperature could reduce DNA synthesis.

But in this assay, the authors actually measured the rectal temperature and found out that for the three isomers out of the four they tested, the drop in rectal temperature alone did not account for the observed reduction of the DNA synthesis in the case for 2,4, 2,5, and 3,4-isomers.

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DR. SUN: So we identified three review papers that evaluated this assay. The Donatsch 1982 paper, they tested 10 chemicals, four mutagens, six non-mutagens, and three out of the four mutagens tested positive in this assay. Six out of six non-mutagens also tested positive. These are the false positives. So their conclusion is this assay is not reliable unless it's tested under isothermal conditions.

In another paper, Seiler 1977, tested 100 chemicals. And their true positive rate is 86 percent. And their false positive rate is 10 percent. So the point -- concern is the low sensitivity of this assay.

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DR. SUN: The last one, Lambert and Erikkson,

they tested 12 chemotherapeutic drugs, and their conclusion of this assay is it's a good preliminary tool for detecting genotoxicity, but people cannot rule out the depletion of precursor pool. And they also bring up a way to improve this assay to differentially label the DNA with two kinds of radioisotopes. It would make it a better method.

That's all I have to say.

CHAIRPERSON MACK: Thank you, Dr. Sun.

All right. From what we've heard now in the last half hour, or last -- since we went on, could we go down the line and see if anybody has additional comments.

Dr. Zhang.

COMMITTEE MEMBER ZHANG: What I missed?

COMMITTEE MEMBER EASTMOND: Any additional comments.

COMMITTEE MEMBER ZHANG: No.

CHAIRPERSON MACK: No.

Dr. Dairkee?

COMMITTEE MEMBER DAIRKEE: No.

CHAIRPERSON MACK: David?

COMMITTEE MEMBER EASTMOND: I just said quite a bit. I don't.

CHAIRPERSON MACK: Nothing changed.

Joe.

COMMITTEE MEMBER LANDOLPH: Yeah, I looked -- relooked at the tumor data for the 2,5 and the 2,6, and it's not the most fantastic data on the planet, but these are genotoxic across the spectrum, except for the in vivo. And they are tumorigenic, so I'm going to say about where I was, but that I have looked at that data more carefully.

CHAIRPERSON MACK: Peggy.

COMMITTEE MEMBER REYNOLDS: No.

CHAIRPERSON MACK: Jason?

COMMITTEE MEMBER BUSH: No comment.

CHAIRPERSON MACK: Okay. Then I think we're ready to take a vote.

COMMITTEE MEMBER EASTMOND: Can I make one comment.

CHAIRPERSON MACK: Yes.

COMMITTEE MEMBER EASTMOND: Joe, you do realize the NCI did not consider them tumorigenic?

CHAIRPERSON MACK: Yes, I did.

COMMITTEE MEMBER EASTMOND: Okay. All right.

COMMITTEE MEMBER LANDOLPH: Yeah, I realize that. And I think they, you know, should have done a better job in the first place. But I did re-examine that data and I liked the -- you know, the liver tumor data is convincing and the pancreatic tumor data is convincing. It could always be better, it could be more, but we've got to go

with what we've got.

CHAIRPERSON MACK: I'm a little disturbed by the definition of mixed, because I don't understand whether or not there's any systematic definition. If mixed means 2.4 plus anything else, then I think the judgment should be made on 2.4, not on mixed. But I guess if mixed contains everything else but 2.4, then the issue of genotoxicity becomes really important, or at least what you think is the significance of genotoxicity becomes really important.

But I gather there is no definition, so mixed simply means whatever combination of diaminotoluenes one happens to have in one's hand is mixed. So let's now take a vote.

Has diaminotoluene mixed been clearly thrown -- clearly shown through scientifically valid testing, according to generally accepted principles to cause cancer?

First, let's ask for yes votes. Everybody who considers that these should be listed, please raise their hand.

(No hands raised.)

CHAIRPERSON MACK: That's nice.

Now, let's take no votes. Everybody who believes that diaminotoluenes mixed have not been clearly shown through scientifically valid testing, according to

generally accepted principles to cause cancer? So everybody who believes no, raise their hand.

(Hands raised.)

CHAIRPERSON MACK: Unanimous.

So we will de-list diaminotoluenes mixed.

Now, I guess it's time for lunch. How long a lunch break we want to take?

Oh, we want to -- do we want separate votes on each of them?

ACTING DIRECTOR ZEISE: Yes.

CHAIRPERSON MACK: Okay.

So now let's take votes on -- and I'm going to just ask for no votes and we'll go to yes votes if there's a problem with no votes. Has 2.3-diaminotoluene --

COMMITTEE MEMBER EASTMOND: Yeah. Carol.

CHAIRPERSON MACK: Yes, Carol.

CHIEF COUNSEL MONAHAN-CUMMINGS: So Dr. Mack, maybe you can just start with the yes votes. The same as we normally do, if you could just ask whether or not the chemical has been clearly shown --

CHAIRPERSON MACK: Well, I'll ask for he yes vote afterwards. I'm just assuming that we're going to have unanimity. And if we don't, I'll ask for the yes vote. Is that bother -- does that bother the law?

CHIEF COUNSEL MONAHAN-CUMMINGS: As long you ask

both questions, we'll be okay.

CHAIRPERSON MACK: As long as I what?

CHIEF COUNSEL MONAHAN-CUMMINGS: As long as you ask both questions, yes or no, separately.

CHAIRPERSON MACK: Oh, all right.

(Laughter.)

CHAIRPERSON MACK: Just to make you happy then, we'll go yes first, and we'll look around carefully.

CHIEF COUNSEL MONAHAN-CUMMINGS: Thank you.

CHAIRPERSON MACK: Has diaminotoluene 2.3 -- 2.3-diaminotoluene been clearly shown through scientifically tested, according to generally accepted principles to cause cancer? All of those believe yes, raise their hand.

(No hands raised.)

CHAIRPERSON MACK: All of those who believe no, please raise their hand.

(Hands raised.)

CHAIRPERSON MACK: Unanimous.

Now, we go to 2.5, which is the most interesting one. Has 2.5-diamintoluene been clearly shown through scientifically valid testing, according to generally accepted principles to cause cancer? All believe -- all who believe the answer to that is yes, raise their hand.

(Hands raised.)

CHAIRPERSON MACK: One, two, three. Three and counting.

All believe the answer to that question is no, raise their hand.

(Hands raised.)

CHAIRPERSON MACK: One, two, three, Dr. Zhang, are you voting?

COMMITTEE MEMBER ZHANG: (Shakes head.)

CHAIRPERSON MACK: You're going to obtain.

COMMITTEE MEMBER ZHANG: (Nods head.)

CHAIRPERSON MACK: Well, that puts us in an interesting position. So the result is 3 to 3 with 1 abstention.

All right. Now we go to 2.6. Has 2.6-diamintoluene been clearly shown through scientifically valid testing, according to generally accept principles to cause cancer? All of those believe yes, raise their hand.

(Hands raised.)

CHAIRPERSON MACK: Three.

All of those who believe no, raise their hand.

(Hands raised.)

CHAIRPERSON MACK: Four this time.

Then we go to 3.4. Has 3.4-diamintoluene been clearly shown through scientifically valid testing,

1 according to generally accepted principles to cause
2 cancer? All those who believe yes, raise their hand.
3 (No hands raised.)
4 CHAIRPERSON MACK: All of those who believe no,
5 raise their hand.
6 (Hands raised.)
7 CHAIRPERSON MACK: Unanimous.
8 And finally, to 3.5. Has 3.5-diamintoluene been
9 clearly shown through scientifically valid testing,
10 according to generally accepted principles to cause
11 cancer? All those who believe yes, raise their hand.
12 (No hands raised.)
13 CHAIRPERSON MACK: And all those who believe no,
14 raise their hand.
15 (Hands raised.)
16 CHAIRPERSON MACK: Unanimous.
17 ACTING DIRECTOR ZEISE: That's it. Okay. That
18 makes it time for lunch.
19 CHAIRPERSON MACK: Yes, Carol, what did I do
20 wrong.
21 CHIEF COUNSEL MONAHAN-CUMMINGS: No, you're --
22 that's fabulous. Thank you, Dr. Mack. I just want to
23 remind everyone that there's still issues in front of the
24 Committee. And so while you're at lunch, if you could not
25 speak to yourselves or others about the chemicals that

we're talking about this afternoon, I'd appreciate it.
Thank you.
(Off record: 12:27 PM)
(Thereupon a lunch break was taken.)

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

(On record: 1:39 PM)

CHAIRPERSON MACK: Let's begin again. Go ahead.

(Thereupon an overhead presentation was presented as follows.)

DR. SANDY: Gwen -- Dr. Gwen Osborne will be presenting this HID.

DR. OSBORNE: Good afternoon. My name is Gwen.

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DR. OSBORNE: And I'm going to be presenting the data on nitrapyrin, which is a chemical listed as causing cancer by the U.S. EPA and under review by the CIC now.

In 2000, U.S. EPA formally identified nitrapyrin as likely to be carcinogenic in humans. Then in 2005, it was listed under Proposition 65 as causing cancer through the authoritative bodies mechanism. In 2012, U.S. EPA reclassified it as suggestive evidence of carcinogenic potential. And nitrapyrin has not been reviewed by any other agencies.

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DR. OSBORNE: So here's an outline of the presentation today. I'm going to start with the chemical identity use and occurrence, go through the carcinogenicity evidence with the animal cancer bioassays and other relevant data, discuss the possible carcinogenic

mechanisms, and end with a summary of the data.

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DR. OSBORNE: So here we have the chemical structure of nitrapyrin. It's full name is 2-Chloro-6-(trichloromethyl)pyridine.

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DR. OSBORNE: Nitrapyrin is a pesticide that functions as a bactericide and nitrification inhibitor. It's registered for use on corn, wheat, cotton, sorghum, strawberries, and sudangrass in California. It has not been detected in ground or surface water, and has never been detected in foods tested by pesticide residue monitoring programs in California or by the U.S. FDA. So the potential for dietary exposure is considered low, but agricultural workers may be exposed.

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DR. OSBORNE: No epidemiological studies on the effects of human exposure were identified. The carcinogenicity has been studied in rats and mice. There are two unpublished dietary studies in Fischer 344 rats, and four unpublished dietary studies in B6C3F1 mice.

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DR. OSBORNE: In the rat studies, nitrapyrin was administered in the diet to groups of 50 male and 50 female rats at doses of 0, 5, 20, or 60 milligrams per

kilogram body weight per day for two years. This table shows the incidences of kidney tumors in male rats with the denominator representing the effective tumor numbers.

There are three renal tubule adenomas and three adenocarcinomas in the high dose group, which is significantly increased when they were combined. This combined incidence exceeded laboratory historical control data. And these tumors are considered rare in male F344 rats. No treatment related tumors were observed in female rats.

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DR. OSBORNE: Four feeding studies were conducted in B6C3F1 mice, with 50 male and -- sorry, 50 male and 50 female mice per dose group.

Quast et al., 1990 was conducted at lower doses of 5, 25, or 75 milligrams. No treatment related tumors were observed in these studies. In the higher dose studies by Stebbins and Cosse, 1997, doses were increased to 125 and 250 milligrams.

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DR. OSBORNE: This table shows the tumors observed in male mice in the higher dose study. Statistically significant increases in liver adenomas and combined adenomas and carcinomas were seen in the high dose group with significant dose response trends.

Forestomach squamous cell papillomas and combined papillomas and carcinomas were also significantly increased at both the mid and high dose groups with significant trends. Forestomach carcinomas are considered rare in male B6C3F1 mice.

Tumors of the epididymis were also observed in both treatment groups and significantly increased in the high dose. The original study classified these tumors as malignant, undifferentiated epidiymal sarcomas. The 2000 and 2005 EPA reviews called the these epidiymal tumors in most testes and were considered treatment related.

In 2010, a pathology working group review sponsored by the registrant reclassified these tumors as histiocytic sarcomas. The 2012 EPA review then considered these tumors to be not treatment related, in part because the registrant suggested the tumor incidences for the lower dose study and high dose study be combined.

U.S. EPA's consulting pathologist stated with the data from the two studies combined, it is clear that the occurrence epididymal histiocytic sarcoma is incidental not related to treatment. However it is not accepted practice to combine controls, because the studies were conducted seven years apart.

U.S. EPA guidelines for carcinogen risk assessment states that the most relevant historical data

come from the same laboratory and are gathered within two or three years, one way or the other, of the study under review. Other data should be used only with extreme caution.

When the EPA reviewed the nitrapyrin study in 2012, they also said that incidences of epidiymal tumors were within historical control ranges. They cited an incidence of 0.5 percent of histiocytic sarcoma in NTP historical controls with a range of 0 to 4 percent.

However, the tumor incidence observed in the high dose group of this study was actually greater than four percent. Plus, this historical data -- control data is for histiocytic sarcomas observed at all sites. Histiocytic sarcomas specifically in the epididymis are considered rare in mice.

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DR. OSBORNE: Now, this table shows the tumor incidences in female mice for the higher dose study. We see increases in liver adenomas and combined adenomas and carcinomas at both the mid and high dose levels with -- both with significant trends.

Incidences of forestomach papillomas and combined papillomas and carcinomas were also increased at both dose levels with positive trends. Forestomach carcinomas are also considered rare in female mice.

Finally, there was an increase in harderian gland adenomas. In both the mid and high dose groups, the incidences of these tumors were above the control incidence and more than four-fold above the lab historical control incidence that the 2000 EPA review cited a 3.5 percent.

The registrant suggested that the low and high dose studies be combined for these studies -- for these tumors. U.S. EPA did not combine the studies, but said the control for the second study is lower relative to the first. But again, it's not accepted practice to compare these studies, because they were conducted seven years apart.

U.S. EPA guidelines for carcinogen risk assessment also indicate that tumors should not be discounted because incidence rates in the historical -- in the concurrent controls are lower than average or because incidence rates are within the range of historical controls.

I would also like to note that the Stebbins and Cosse study report listed the historical control incidences of harderian gland tumors in their report, and that these incidents are from studies conducted in the same lab from 1983 to 1995. And they combined harderian gland adenomas and carcinomas. When we looked at the

appropriate years, the incidences range from 6 to 10 percent in female mice.

In mice treated with nitrapyrin, the tumor incidence is 17 percent in the mid dose group and 19 percent in the high dose group.

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DR. OSBORNE: So that concludes the carcinogenicity data that we identified. And now I'm going to move on to the pharmacokinetics and metabolism. The pharmacokinetics of nitrapyrin have not been studied in humans, but have been studied in rats, mice, goats, chickens, and dogs in feeding studies and in one dermal absorption study in rats.

Nitrapyrin is rapidly absorbed and distributed throughout the body and does not accumulate. Metabolites are quickly eliminated primarily in the urine.

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DR. OSBORNE: Here is the proposed metabolic pathway. Both rats and mice metabolize nitrapyrin as 6-chloropicolinic acid or 6-CPA, and then to its glycine conjugate. In mice, a taurine conjugate has also been detected.

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DR. OSBORNE: So moving on to the genotoxicity data. Nitrapyrin was tested in a limited number of

assays. It was tested in salmonella in three studies, two of which were positive and one negative. Nitrapyrin did not induce mutations in other genotoxicity studies.

U.S. EPA considered that there was -- said that there was a concern for mutagenicity in the 1992, 2000, and 2005 reviews. In 2012, this was changed to no concern, in part because of a state -- a report stating that the differences in the results of the salmonella assay were due to different criteria being used.

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DR. OSBORNE: So I want to take a moment to talk about the salmonella studies. Different criteria were used to evaluate the different studies. A criteria used by the Zeiger et al. 1998 study are shown here. Individual trial were judged depending on the magnitude of the increase of revertants and the shape of the dose response. They added that it was not necessary for a response to reach two-fold over background for a chemical to be judged mutagenic.

Other studies use different criteria. The salmonella study by Mecchi in 2007 required a minimum fold increase for a test to be considered positive. A lot of work has been done over the decades on the evaluation of genotoxicity, and the issue of requiring a two- or three-fold change for positive evaluation has arisen a

number of times. For example, two references included in the hazard identification materials by Mortelmans and Zeiger in 2000 and Kim and Margolin in 1999, have discussed the weaknesses of using fold cutoffs. Two issues are that it does not have validity as a decision rule, and it may be too conservative.

Therefore, we found it appropriate to judge compounds as mutagenic, if they demonstrated a concentration related and reproducible increase of revertants according to the generally accepted criteria.

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DR. OSBORNE: Here are the three salmonella assays with the results for the various strains. On the left is Kennelly, 1985, which used the plate incorporation protocol. These results were reported as negative.

Then in the middle is the study by Zeiger et al., in 1988, and was conducted using the pre-incubation method. Results presented here are assessments made by the study authors based on the criteria in the previous slide. Positive responses were observed in Salmonella strain TA97, 98, and 100 with both rat and hamster liver S-9 metabolic activation. Details of that study are presented in Tables 9, 10, and 11 of the document.

The third study on the right was conducted by Mecchi in 2007, also using a pre-incubation protocol. I

want to take a moment to walk you through these results. When there is a plus and minus, the plus indicates that dose related reproducible positive responses were observed when the criterion on the last slide were used, while the minus indicates that the study author considered the test to be negative when using the two- or three-fold criteria.

The author did note that increases were seen in strains TA98 and 100, but they did not meet the fold requirements.

So strain TA98 was positive with and without metabolic activation. Footnote 1 indicates that increases were seen in the follow-up study with activation and Footnote 2 shows that the initial follow-up studies were positive without activation. Footnote 3 shows that EPA considered this a weak positive.

Strain TA 100 was positive in both studies with metabolic activation, and in the follow-up study without activation. And finally, strain TA1535 was positive in both studies with activation. Details of this study are represented in Tables 12 and 13 of the document, if you want to look more closely.

I would also like to note that the pre-incubation protocol is generally more sensitive than the plate incorporation protocol. Also, hamster liver S-9 fraction may be more sensitive than rat for metabolic activation in

some compounds.

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DR. OSBORNE: So then nitrapyrin was compared to 11 structurally similar compounds. Criteria were -- used to select them were pyridine ring with aromatic chlorine and chloromethyl substitutions and the availability of genotoxicity data.

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DR. OSBORNE: These are the 11 selected compounds with nitrapyrin in the upper left corner.

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DR. OSBORNE: This table shows the available data for those comparison compounds. Six of them induce mutations in salmonella and/or the mouse lymphoma cell assay. One of these, pyridine is listed as a carcinogen under Proposition 65. It induced tumors in mice at some of the same sites as nitrapyrin, including the mouse liver and kidney.

3-(chlorolmethyl)pyridine also induced tumors at some of the -- at a similar site in the forestomach. 2-(chloromethyl)pyridine did not induce tumors and the rest of these compounds have not been tested.

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DR. OSBORNE: We also investigated high throughput screening data. Nitrapyrin has been tested in

403 assays in the U.S. EPA ToxCast database, and was active in seven. Five of the active assays detect upregulation of transcription factor activity, and two detect downregulation of chemokine gene expression.

Analysis using the comparative toxicogenomic database indicates that five of these target genes have been associated with cancer pathways, which are the ones highlighted in red.

So this gives us some insight, but doesn't really tell us much about how nitrapyrin is actually working.

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DR. OSBORNE: The mechanisms through which nitrapyrin induces tumors are not known. A number of hypotheses have been suggested these are genotoxic, alpha2u-globulin nephropathy associate kidney tumors, cytotoxicity, and activation of constitutive androstane receptors or CAR.

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DR. OSBORNE: As discussed earlier, a concentration related and reproducible increase was observed in four strains of salmonella in two studies that used the pre-incubation method, a negative using the plate incorporate method -- protocol and in other mutagenicity assays.

Guidance has been developed to assess the overall

mutagenicity of a chemical and direct further testing. The handbook of carcinogenic potency and genotoxicity database states a chemical is designated non-mutagenic only after it had been tested in at least four strains without activation and with rat and hamster S-9. A positive result in one strain with one type of metabolic activation was sufficient to identify a chemical as a mutagen.

Guidance developed by the International Association of Environmental Mutagen Societies states that negative in vivo tests do not overrule positive in vitro tests because they may have different sensitivities or evaluate different endpoints. A positive in vitro result is not automatically overruled, and some follow-up testing or investigation is generally necessary to determine the relevance of the in vitro positive result.

But nitrapyrin has not been adequately tested for other genotoxicity endpoints. For example, it has not been tested for induction of oxidative DNA damage or DNA single-strand breaks or in several other tests.

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DR. OSBORNE: Nitrapyrin induced kidney tumors in male rats. And it was proposed that the mechanism for these tumors is through male rats, specific alpha 2u-globulin nephropathy.

This slide shows IARC's criteria for determining whether a chemical causes kidney tumors through this mechanism. OEHHA's assessment of the available data found that two of these criteria were met. The identification of the protein as alpha-2U-globulin, and similarities in the dose response relationship of the tumors with the histopathological endpoints.

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DR. OSBORNE: We determined that two of the criteria were not met. There were positive genotoxicity results, so criterion one was not met, and criterion two was not met because effects were observed in female rat kidneys and a rare rat -- a rare kidney tumor was seen in male mice in each of the two dose groups in the low dose study, and increases in kidney weights were seen in the higher dose mouse study. Criteria 3, 5, and 6 were not able to be evaluated because there was no data.

It is also interesting that a structurally similar compound, pyridine, was found to induce kidney tumors in male rats in a study by NTP. Some changes were consistent with the alpha-2U-Globulin response, but NTP concluded that these tumors were not attributable to alpha-2U-Globulin.

Given that some changes were seen in a similar compound but were not considered to be induced solely by

alpha-2U-Globulin other mechanisms may contribute to the induction of kidney tumors by nitrapyrin.

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DR. OSBORNE: Prior to 2005, the registrant proposed that nitrapyrin induces liver tumors through a cytotoxic mechanism. In 2005, U.S. EPA determined that there were not enough data to support this hypothesis, and the registrant agreed, and in 2012 the U.S. EPA added that there is no clear indication of hyperplasia or necrosis.

In a recent report, the registrant was in agreement with this and stated that cytotoxicity is not likely a mechanism for liver tumors.

The registrant also proposed that forestomach tumors in mice were due to irritation of the forestomach. However, U.S. EPA noted that nitrapyrin does not seem to be unusually more irritating than other chemicals that do not produce forestomach tumors.

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DR. OSBORNE: The last mechanism proposed was constitutive androstane receptor activation, or CAR, that leads to formation of liver tumors. For a little background, CAR is a transcription factor that regulates the expression of many genes involved in the metabolism and transport of chemicals in humans and rodents. Once activated, CAR upregulates the Cytochrome P450 2B gene

family.

Phenobarbital is a prototypical inducer of hepatic CYP2B enzymes in humans, rats, and mice, and has been studied in rodents as a possible model for understanding CAR activation in liver tumors.

CAR mediated signaling is very complex, because of the overlapping CAR and PXR ligand specificities and intricate cross talk with other transcription factors.

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DR. OSBORNE: Several key events are required for the proposed CAR mode of action. According to U.S. EPA, these are CAR activation increased CYP2B10 expression, PROD activity, liver hypertrophy, cell proliferation, increase liver weights, basophilic foci, and induction of liver tumors.

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DR. OSBORNE: I'm going to go through the data for each of these key events, but first I want to give you EPA's overall view of this proposed mechanism in 2012.

After reviewing the mechanistic studies available at the time, they concluded that while there's some evidence of CAR activation, this finding was not supported by key data on P450 and specific enzyme induction. Also, the cell proliferation data did not show the typical profile that U.S. EPA expects with a CAR inducer.

It was concluded that the available data did not adequately support a CAR mode of action, and U.S. EPA then recommended that cell proliferation studies be done earlier in the course of treatment and that a CAR null mouse assay be considered.

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DR. OSBORNE: In response to that request, more studies were submitted by the registrant. I'm going to discuss the results in detail on the following slides. But to give you some context, in the studies reviewed in 2012, there was no PROD activity, which is a functional measure of the CYP2B10 enzyme, and there were no increases in total P450 protein content.

The registrant suggested that nitrapyrin induces suicide inhibition of the PROD enzyme. To test this an in vitro experiment was done in phenobarbital-induced liver microsomes. So to test this, an in vitro experiment was done in phenobarbital induced liver microsomes, nitrapyrin in a positive control inhibited PROD activity, while phenobarbital, as the negative control, had no effect.

The authors proposed that nitrapyrin irreversibly inhibited CYP2B10 mediated PROD activity, which is why it is not seen in the earlier study.

The second study here compared liver cell proliferation in mouse and human cells in vitro.

Nitrapyrin exposure increased the proliferation of mouse hepatocytes, but didn't have an effect on the proliferation of human hepatocytes.

The third study submitted by the registrant after the EPA 2012 review was a four-day CAR knockout mouse study. Several effects were seen in both wild-type and CAR knockout mice, including hypertrophy, increased liver weights, and induction of CYP1A1 3a11, and 4a10.

CAR knockout mice did not demonstrate hepatocellular proliferation or large increases in CYP2B10 gene expression. But the effects -- but the liver changes seen in both CAR knockout and wild-type mice show that nitrapyrin has effects on the liver independent of CAR activation.

Now, I'm going to go through each of the key events with the supporting data.

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DR. OSBORNE: The following slides show simplified versions of these tables and more detailed versions are available in the document. Most of the mechanistic studies were done only in male mice unless otherwise noted, but I would like to note that females actually had a more sensitive response, because liver tumors were induced at lower doses in females than males.

Additionally, the doses used in the mechanistic

1 studies were not the same as the doses used in the
2 long-term carcinogenicity studies. These mechanistic
3 studies were done at 75 milligrams and then jumped to 250
4 and 400, which is different from the two-year study that I
5 presented earlier that used doses of 125 and 250. But we
6 saw liver tumors in female mice at 125, but this dose was
7 not used in the mechanistic studies.
8 So returning to the key events. The first key
9 event is CAR activation, and is demonstrated by induction
10 of CYP2B10. This study also quantified gene expression of
11 CYP1A1, which signals activation of aryl hydrocarbon
12 receptor; 3A11, which signals pregnane X receptor; and
13 4A10, which signals PPAR-alpha.
14 The rows highlighted now are data from the
15 studies reviewed in 2012. We can see that nitrapyrin
16 greatly induces CYP2B10 gene expression especially at
17 doses of 250 milligrams or greater. We also see induction
18 of 1A1, 3A11, and 4A10, although at lower levels than
19 2B10.
20 The study submitted after the 2000 review are
21 consistent with this and show a higher induction of 2B10
22 and lower induction of the other genes. Also, even though
23 CAR was knocked out, you still see induction of 2B10,
24 although it's smaller than in wild-type mice. You also
25 see induction of 3A11 and 4A10 comparable to wild-type

mice.

Overall, these studies indicate that nitrapyrin activates CAR and other nuclear receptors.

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DR. OSBORNE: Key event number 3 is an increase in PROD activity, which is used as a functional measure of the CYP2B10 enzyme and is characteristic of CAR activation. There were no increases in PROD activity after 14 days of exposure to nitrapyrin. There were also no obvious differences in total P450 protein content with only a 1.3-fold change in the high dose groups. It's also interesting that the fold change is 1.5 after a 21-day recovery period, which is not what we would expect in total protein content.

In reviewing these data, U.S. EPA stated that the absence of hepatic metabolic enzyme activity leaves a major uncertainty in the mode of action analysis.

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DR. OSBORNE: The fourth key event is the hepatocellular hypertrophy. This occurred in the majority of mice. In the two-year study included in the 2012 review, hypertrophy was seen in most of the male mice treated with nitrapyrin for one or two years. The newer studies are consistent with this, and all mice treated with at least 250 milligrams demonstrated hypertrophy,

except for the mice allowed a recovery period.

Also, there were no differences in the hypertrophy responses between wild-type and mice with CAR knocked out.

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DR. OSBORNE: Cell proliferation is the fifth key event. In general, it was observed in mice exposed to at least 250 milligrams for all time points, an increase in proliferation was observed in CAR knockout mice treated for four days. I would also like to add that it seems like males and females have different short-term liver responses. Although it's not shown here, proliferation was not seen in female mice at doses that induced tumors. So it seems that other mechanisms are involved in liver tumor development.

As mentioned before, U.S. EPA stated that the cell proliferation response was not what was expected. They usually observed proliferation within one to four days, which declines by day seven. In studies conducted after that 2012 review, an increase in proliferation was seen after four days of nitrapyrin exposure, but it's unusual that we also see proliferation at the end of the one-year study.

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DR. OSBORNE: Increased liver weight is the sixth

key event which accompanies hepatocellular hypertrophy. Overall, increases in liver weights generally mirrored hypertrophy in mice exposed to at least 250 milligrams.

Liver hypertrophy and increased liver weights were observed in CAR knockout mice, similar to wild-type mice. So it appears that these particular liver changes are not mediated solely via CAR.

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DR. OSBORNE: The final key events are an increase in basophilic foci and liver tumors. The data for both of these key events are shown here from the high dose two-year study in male and female mice.

The increase in basophilic foci was significant at 250 milligrams for both sexes. However, the data do not show a correlation between the number of foci and tumor incidents like we would expect. Fewer nitrapyrin treated mice had foci than had liver tumors, in both male and female studies. In the female mouse study, a significant increase in liver tumors was seen at the low dose, but a corresponding increase in foci in that dose group is clearly lacking.

So that concludes our discussion of the key events. Some of these key events were observed in the nitrapyrin studies, but some were not observed or were not observed as we would expect.

The nitrapyrin studies are limited in scope and provide limited information on the mechanisms through which nitrapyrin induces liver tumors.

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DR. OSBORNE: I would also like to point out two studies showing that human and mouse responses phenobarbital may not be drastically different from one another. Luisier et el., 2014 used mice expressing human CAR and PXR, mice lacking both receptors, and wild-type mouse to look at responses after 90 days of phenobarbital exposure to see if humanized mice are responding similarly to wild-type mice.

Humanized mice did respond similarly in both humanized and wild-type induced genes, consistent with hepatocellular proliferation. These genes -- these included genes associated with DNA replication, cell cycle mitosis, and the proliferation related nuclear antigen. These data suggest that the activation of both mouse and human CAR by phenobarbital leads to similar proliferative transcriptional responses.

In another study, a single dose of n-nitrosodiethylamine, or DEN, was given as the initiator to male mice expressing human CAR and PXR followed by promotion with phenobarbital for 40 weeks.

On the left of the chart are wild-type and

humanized mice given only initiator. Forty-seven percent of the wild-type and 80 percent of the humanized mice developed liver tumors. On the right are mice that were given both -- that were then given phenobarbital as a promoter. And you can see that 100 percent of both wild-type and humanized mice developed liver adenomas.

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DR. OSBORNE: So to summarize the data, several tumor types were observed in dietary studies in mice. There were increases in liver adenomas and combined adenomas and carcinomas in the high dose group in males and mid and high dose groups in females.

Increases were also seen in forestomach papillomas and combined papillomas and carcinomas in the mid and high dose groups in males and females. Forestomach carcinomas are considered rare in male and female mice. An increase in rare epidiymal histiocytic sarcomas was observed in male mice. And finally, an increase harderian glands adenomas was seen in female mice. In rats, significant increases were observed in combined renal tubule adenomas and adenocarcinomas.

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DR. OSBORNE: And to summarize the other relevant data, a concentration related and reproducible increase was observed in salmonella in two studies that used the

pre-incubation method. The third study using the plate incorporation method was negative, and other mutagenicity tests were negative, but limited endpoints were tested.

Nitrapyrin activated CAR and study results suggests that it also activates AHR, PXR, and PPAR-alpha. Alpha-2U-Globulin accumulation was proposed as a possible mechanism for kidney tumor development in male rats. Two IARC criteria were met, three criteria lacked data, and two criteria were not fulfilled.

A structurally similar compound, pyridine, induced some changes consistent with alpha-2u-Globulin, but NTP concluded that those tumors were not attributable to alpha-2u-globulin.

Finally, structure activity comparisons indicates some similarities in biological activity. Pyridine and 3-(chloromethylpyridine) induce tumors at some of the same sites as nitrapyrin and 6 of the 11 structurally related compounds are genotoxic.

So that concludes our presentation of the data on nitrapyrin.

Thank you.

CHAIRPERSON MACK: Okay. What do you think?

COMMITTEE MEMBER LANDOLPH: I think your write-up on your presentation was excellent, and so was the write-up in the presentation by the two previous authors.

It's a very interesting compound. And clearly there is some genotoxicity, although there's some controversy over, you know, you have -- it looks like you have to use the plate incorporation, which is more sensitive than the regular incubation.

It's got parallels to pyridine, which it's derived from. I note one of the metabolites is 6-chloropicolinic acid. And I note that picolinic acid used with chromium was given to people as a supplement, and everybody thought it was the chromium that was breaking chromosomes. It turned out it was the picolinic acid. And I was looking to see -- I didn't see any chromosome breakage assays here. So maybe that's something that should have been done.

I find this compound very interesting. The mechanism is clearly very complex, not well understood. I like the animal tumor database, because there's mouse liver, testes, epididymis, forestomach, harderian gland and rat kidney. There's some controversy over whether the mouse liver studies are relevant to human liver carcinogenesis or not.

And there's controversy about the forestomach as to whether that's relevant to humans or not, and some controversy about the harderian glands, which is a unique type of eye tumor of one of the membranes there, and

whether that's relevant. And some controversy about the kidney, but that's one, two, three, four, five tumor sites. So this compound is inducing a heck of a lot of tumors in animals.

And it's genotoxic doesn't -- it's not incredibly genotoxic, but it is so, and it is an animal carcinogen. And it's got relevance to pyridine, which interestingly doesn't seem to have that same genotoxicity, but it does have some of the same tumor sites, and to 3(chloromethyl)pyridine. So I think it's very interesting.

It puts us in a difficult situation, because EPA previously called it a B2 carcinogen, which is likely, which equates to the IARC 2A probable human carcinogen and. Now they're going backwards and lowering it to likely, which I equate to the IARC 2B, which is maybe between 2B and 2A as suggestive, I guess is what they're using, as opposed to possible.

So it puts us in a position of if they delist it, then we delist it, because they delisted it, or do we probably more likely come up with our own independent assessment. I think there's enough tumor database here. Although, there are questions about some of the endpoints and irrelevance to humans that I would tend, even with some of the deficiencies in the tumor database, to rank

this as a human carcinogen and leave it sit as it is as a carcinogen.

CHAIRPERSON MACK: Okay. Let's go down the list starting with you, Jason.

COMMITTEE MEMBER BUSH: All right. Thank you. So I reviewed the hazard information materials. I do thank the OEHHA Reproductive and Cancer Hazard Assessment team for their comprehensive report. Good job.

I reviewed the public comments, documents submitted by the registrant Dow AgroSciences. I reviewed the additional confidential studies submitted by the registrant, which are informative, but because of the lack of formal peer review and unblemished nature, are less significant in my weighting of the evidence.

Starting with any epidemiological studies, since there aren't any, you know, that lack of direct data means that we need to go to other sorts of outcomes. So specifically, some of this is going to be repeating what Joe said, but looking at the genotoxicity data, any animal model surrogates and any mechanistic insights.

With Joe, I agree that the genotoxicity is weak. There's a broad range of endpoints, in vitro bioassays that, you know, I think collectively indicate that nitrapyrin and its primary metabolites don't seem to possess any direct genotoxic activity. There seems to be

1 some confusion and controversy over some of the
2 methodological experimentation with the mutagenicity
3 studies that suggest perhaps a weak, if any, mutagenic
4 potential, probably not significant in real world
5 exposures. Although the rapid dermal absorption of this
6 chemical is a little bit disconcerting, given some of the
7 information that alluded to possible exposure by handlers
8 of this material in the agricultural field.
9 So that leaves us more or less with the animal
10 carcinogenicity data. I agree there's a broad range of
11 animal studies, you know, exclusively on rat and mouse
12 models that do have a broad range of tumors, which is
13 disconcerting, you know, of course, at high doses, but
14 still that is something to be for me to be concerned
15 about.
16 Looking into the epididymal and harderian gland
17 tumors and taking into account what the U.S. EPA has
18 indicated, I think for the most part they do seem rather
19 artifactual. And I agree that they're probably not
20 treatment related. I think more compelling are the
21 forestomach, kidney, and liver tumor burdens. Forestomach
22 lesions have been refuted by the registrant as secondary
23 irritation and not considered relevant to humans.
24 But their own pathology working group stated that
25 it was, and I quote, "Probably represent a continuum in

the development of treatment related hyperplasia". So this suggests to me that it's possible mucosal or squamous cell target tissues implicating nitrapyrin as a possible tumor promoter, rather than initiator, which again is disconcerting, particularly looking at the possible human dermal absorption routes.

The nitrapyrin induced kidney tumors, specifically the renal tubule cell adenomas and adenocarcinomas were increased. The registrant attempts to placate the data by indicating an alpha-2u-globulin mechanism that -- and that that isn't relevant to human risk, but since a majority of human kidney cancer is actually derived from renal tubules, I think that that's a concern regardless of the carcinogenic mechanism.

Liver tumors were increased in a dose responsive manner. The registrant submitted several studies focused on the specific CAR mode of action that the OEHHA team has talked about. You know, induction of CAR and the subsequent CYP2B10 activity and then they go on to suggest, of course, that this is not relevant to human liver cancer risk. And I agree with the U.S. EPA and the reproductive cancer assessment team that there must be some other kind of CAR independent mechanism occurring, particularly when we consider that the PPAR-alpha, PXR, found through the ToxCast database are potentially

contributing to this hepatocarcinogenesis, as well as the indications for elevated -- other elevated P450 isozymes.

And furthermore, it actually leaves me questioning whether there may be some bias in their experimental approaches. It seemed like they were trying to establish CAR as this sole indication or sole mechanism, which I think would be problematic.

The registrant claims in the public comments document to, and I quote, "Definitively evaluate human relevance for the nitrapyrin specific response in vitro studies evaluating the proliferative response of primary mouse and human hepatocytes to nitrapyrin were conducted.

I had a look at the data through the confidential study that we were given. They refer to, and sorry -- when I delved into that, it seemed to me that there were several experimental flaws with some of the DNA synthesis studies only being counted by a single user. There are errors in standard deviation, or lack thereof, and I think that certainly detracted from any impact from their conclusions in that accompanying document that they sent.

I do agree that what is lacking here is some metabolic studies of any kind. I didn't see anything like that. So any kind of metabolic studies in human tissue surrogates would certainly, you know, help, I suppose, with trying to get nitrapyrin delisted.

The -- I did find compelling the structure activity relationships, anything -- you know, the biological activity, the tumor burden similarities with pyridine is definitely persuasive for me.

So in summary, I concur with the U.S. EPA 2012 decision that additional data over the past 10 years do support a downgrading as to suggestive evidence of carcinogenic potential to humans to that category. But since our statute is less restrictive and our mandate stipulates specifically to cause cancer irrespective of the system, my conclusion is that the weight of the evidence, while getting more convincing over the last several years in the absence of further human specific data, does not warrant delisting.

CHAIRPERSON MACK: Thank you, Jason.

Dr. Reynolds.

COMMITTEE MEMBER REYNOLDS: I don't have anything.

CHAIRPERSON MACK: Dr. Eastmond

COMMITTEE MEMBER EASTMOND: Thank you.

I mean, I think the -- I mean, the evidence is pretty clear cut that this causes cancer in rodents. And the real challenge -- and the only way -- and so it would automatically be listed unless it does so through a mechanism which isn't relevant to humans. And that's

where it becomes a very high burden, because you've got five different tumor types to go with.

And I think the registrants have done a pretty impressive job in, you know, tackling many of these. But it's hard to envision that all of them, you know, are not relevant when you have five different tumor types, and some of them are extremely strongly induced.

I think that OEHHA pointed out effectively our weaknesses in some of these, you know, mechanisms. You would expect these different steps and you don't get all of them. To some degree, that's -- you know, I think you would -- that may be just normal data. You know, things aren't going to be perfect. You're going to have some weaknesses.

But I find it hard simply because, you know, if you go down systematically, there are just enough weaknesses in multiple of these sorts of mechanisms that makes it hard to, you know, to say none of these are relevant to humans, which I think the standard you have to do so. It's tough. For me, it would be hard to buy into that.

CHAIRPERSON MACK: Thank you.

COMMITTEE MEMBER DAIRKEE: Yeah, I concur with everything that's been said. And I do see that the genotoxicity data is weak, but genotoxicity is not the

only way to cancer. And there are the other data that is there, which shows effects on proliferation and those types of processes, make sense, and go a long with why tumor formation occurs in mice.

And I also agree that we have no human information at all about this chemical, but it does sound like it plays a role in cancer.

CHAIRPERSON MACK: Dr. Zhang.

COMMITTEE MEMBER ZHANG: To me, I think the animal carcinogenesis data kind of pretty convincing, you know, multiple tumors in mice and the rats, but, to me, it seems like in the male animal more than the female. This is one thing.

And the genotoxicity, yeah, you know, you just mentioned. I think this is a chemical we may not fully understand the mechanism, but I think that's not the required, right? As long as -- and also, without human data, that's not required. As long as we have the animal carcinogenesis data, I think it's enough.

So I basically I agree with most of the committee members.

CHAIRPERSON MACK: All right. Well you're -- that was what you were trying to tell me again.

Okay. It looks like the Dow people are going to have a reasonable tough job. So let's have a go at it.

(Thereupon an overhead presentation was presented as follows.)

DR. LaROCCA: Hi. I'm Jessica LaRocca. I'm a mammalian toxicologist at Dow AgroSciences. And I'd like to express my gratitude for having the opportunity to share with you some of our data.

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DR. LaROCCA: And what I'm going to be going over is just some of the relevant scientific and regulatory history for nitrapyrin, which supports delisting it from Prop 65.

And I'm going to skip over these introductions for what nitrapyrin is to cut down on my time.

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DR. LaROCCA: But it's been registered since the 70s in the U.S., which is supported by a number of studies and independent reviews, both from regulatory agencies as well as experts external to Dow.

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DR. LaROCCA: As far as the regulatory reviews, they're listed up here from the CPRC and the CARC with the most recent review being conducted in 2012 where it was downgraded to suggestive evidence. There's also an upcoming CARC review scheduled for around 2017 where additional data were supplied and an updated human

relevance framework mode of action study, which support further reclassification to not likely. And this is currently under review.

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DR. LaROCCA: As far as the external reviews for the data for nitrapyrin, this includes an expert review from Dr. Errol Zeiger, who independently evaluated the Genotoxic potential of nitrapyrin. There was also a scientific advisory group convened in 2004, which was -- consisted of a group of independent pathologists. And also, a pathology working group was convened in 2010 also consisting of a group of independent expert pathologists.

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DR. LaROCCA: And included in these reviews are the three cancer bioassays, which you've already been introduced to. First, in the rat, where kidney tumors were observed in male mice only due to the alpha-2u-globulin mechanism. And then the first mouse cancer bioassay, which lead to the first classification for nitrapyrin.

However, a maximum tolerated dose wasn't achieved in the first mouse cancer bioassay, so we've repeated that with higher doses at 125 and 250 mg/kg per day. And there were tumors observed in these mice, which lead to classification of likely to be carcinogenic to humans.

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DR. LaROCCA: So for the past decade and a half, we've been investigating all of these relevant endpoints, including the tumors formed both in the mice as well as in the rat, as well as the genotoxic potential, because that can pertain to the carcinogenic potential of this molecule.

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DR. LaROCCA: And beginning with the genotoxicity, these are the data and the tests that we have available for, nitrapyrin, including the three Ames tests, which have been overviewed already. We also have two upper tier level in vitro assays in mammalian systems, the HGPRT and young scheduled DNA synthesis tests, and also two in vivo studies, the mouse bone marrow micronucleus and the mouse liver unscheduled DNA synthesis.

And all of these were clear negatives with the exception of these Ames tests. So I'm just going to briefly show the data in a little bit of a -- in a different view, but restricting it to these two strains just due to the instance of time here.

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DR. LaROCCA: So I'll show you the TA98, which has a low incidence of background revertants. And if you

choose to use the cutoff criteria, It requires a three-fold cutoff. And all of the other strains with a low incidence of background revertants had a lower fold change, so that's why I'm showing you this one.

And I'm also going to show you the TA100 data, which has a higher rate of background revertants, so therefore you'd use a two-fold cutoff criteria instead of the three.

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DR. LaROCCA: And these are all in the press S-9. So in this graph here, we have the positive controls over on the side of the axis to show you what a true positive control will look like for the Ames tests. And as you've heard seen, while these might not meet the two- or three-fold cutoff criteria, you do see a dose dependent increase in the number of mean revertants, particularly at doses that were above the level of cytotoxicity in other studies, which was observed in the Dr. Zeiger study, the NTP report. And also, this was more evident in the presence of 30 percent S-9, as opposed to the more typically used 10 percent S-9.

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DR. LaROCCA: And moving on to the TA100, again you see kind of the same response, where you might not see the two or three fold cut-off criteria. There is a

concentration related increase. But when comparing it to the positive controls, it's really not as robust of a response.

And regardless of the criteria that you used, whether it's a two- or three-fold cutoff or, you know, just a concentration related increase, when you have positive results in conjunction with other negative results, it's important to actually follow up with this with higher tiered tests, both in in vitro studies as well as in vivo studies.

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DR. LaROCCA: And what we have here are again the HGPRT study where we have no induction of mutations following nitrapyrin exposure. The in vitro unscheduled DNA synthesis tests where we have no increase in unscheduled DNA synthesis.

We also have two in vivo studies. And I think these are the most compelling. The first with the mouse bone marrow micronucleus, where we have clear negative results, as well as the mouse liver unscheduled DNA synthesis tests.

And when performing these second tier studies in in vivo systems, it's important to conduct these in relevant tissues. So that's why the liver was chosen here, because we know that it causes liver tumors.

And I'd also like to point out, earlier I mentioned that Dr. Zeiger, who is the author of the NTP report where you see that weak positive response, he evaluated all of this data for nitrapyrin and he concluded that there's no concern for mutagenic mode of action.

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DR. LaROCCA: So taking into account the guidance that we have available from the EPA, as well as the WHO, this supports that a single weak positive is not sufficient to ascribe a concern for in vivo mutagenicity --

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DR. LaROCCA: -- which is in conjunction with our conclusions, as well as with the external expert, Dr. Errol Zeiger. Again, he was the author of that weak positive report for nitrapyrin, as well as the decision made in 2012 with the U.S. EPA.

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DR. LaROCCA: So moving on to the forestomach neoplasms. These were observed at the two highest doses in the second mouse cancer bioassay, and these were thought to occur secondary to a local irritation effect. And the data that we have for nitrapyrin to suggest that is with two acute studies, one in the eye, as well as in dermal irritation where it is moderately irritating.

And because of structural and physiologic differences between mice and humans, in the sense that mice have forestomachs and humans do not, and forestomach is a storage organ, These tumors are generally considered to be not relevant to humans.

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DR. LaROCCA: However, when you take into account IARC criteria, which states that, you know, while we do not have a forestomach, we do have comparable squamous epithelial tissue, such as in the oral cavity or in the esophagus. So therefore, in principle, they could be considered to be relevant for humans.

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DR. LaROCCA: So taking into account the data that we have available for nitrapyrin is that following chronic exposure, we have no evidence of irritation hyperplasia, or neoplasia in the oral cavity or the esophagus in mice.

Again, the forestomach is a storage organ, so because of this, the exposure time to this tissue is going to be considerably longer than that for the other tissue, such as with the esophagus. And again, there are qualitative differences between the forestomach of the mouse, which has the squamous epithelial tissue, as opposed to the human stomach, which is not.

And therefore, when taking this data into account, a local disposition of nitrapyrin needed to result in a cancer response in the epithelium would not be achievable in humans as compared to the rat, which was the conclusions of the 2004 scientific advisory group, which consisted of the group of independent pathologists.

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DR. LaROCCA: And this supports our conclusions that the forestomach lesions that occur secondary to irritation are not relevant for human health risk assessment, which was validated by the U.S. EPA CARC conclusions in 2005 and 2012.

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DR. LaROCCA: So moving on to the liver tumors, and I'll spend a little bit more time on these. Again, these were observed at the two highest doses in the second mouse cancer bioassay. And because of this, we spent several years embarking on a number of studies to evaluate a potential mode of action, as well as evaluate what the relevance is for humans.

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DR. LaROCCA: And overall, we have the data to support that it is CAR activation, that's the mode of action, which is characterized by the key events that you've already been shown, which is CAR activation, as

well as an increase in hepatocellular proliferation. We also have data to support that this is the mode of action, and alternatives can be excluded based on coherence or plausibility. And we also have data specific for nitrapyrin showing that this is not a relevant mechanism for humans.

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DR. LaROCCA: So going over our study design, mice were exposed to 0, 75, 250, or 400 mg/Kg of nitrapyrin, with those two highest doses corresponding to the carcinogenic dose or above. And the time points that we have here are four, seven, and 14 days. Originally, we only had and seven and 14, but in the last three years, we incorporated an additional time point with the four days of exposure, which address those EPA CARC concerns, which were talked about earlier.

And the endpoints that we evaluated include the gene expression of CYP2B10, the enzyme activity PROD, as well as liver weight increases, hypertrophy, and proliferation

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DR. LaROCCA: And beginning with activation of CAR the CYP2B10, here you can see that following exposure at and above the carcinogenic dose, we have a clear robust increase in CYP2B10. And when you take nitrapyrin away

and not allow these animals to recover, this treatment effect is recovered. And this is characteristic for this mode of action.

We also see similar responses for liver weight increases and hypertrophy, but I'm not going to show you those graphs because we're short on time.

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DR. LaROCCA: So we typically expect to see with CYP2B10 and CAR activation with phenobarbital is CAR activation activates downstream genes and pathways, one of which is CYP2B10 which is a biomarker. And here what you typically see is PROD is converted by CYP2B10 into resorufin, and this what you would measure in your assay. But as you know, when we did that, we see no changes in PROD activity, which was perplexing.

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DR. LaROCCA: So then we went on to look at the protein expression. Well, we know we have the gene, so what's going on with the protein. And when you see with the western blot, we do have an increase in the protein expression, which characterizes the same effects with the gene.

So we step back to think about this. So what could really be going on biologically that could explain this? So we thought about some of the possible

mechanisms, and one of which is suicide inhibition.

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DR. LaROCCA: So an everyday example of this would be grapefruit juice, which is while you'll see on some of your medication bottles, like Lipitor, do not take with grapefruit juice. And the reason for this is grapefruit juice causes suicide inhibition of those enzymes. So please don't take those together.

So what you'll see in the case of suicide inhibition, and what we thought was going on --

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DR. LaROCCA: -- is following activation of CAR by nitrapyrin, this activates downstream genes CYP2B10, which we see with the gene and protein expression. But at the same time, you get inhibition by nitrapyrin, so you don't get that conversion of PROD to resorufin.

And the way that you can assess this is by a pretty simple assay using phenobarbital induced microsomes, which we tested in response to a negative control phenobarbital, a positive control, curcumin, and finally nitrapyrin.

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DR. LaROCCA: And as we expect with our negative and positive controls, with curcumin the positive cool. We see clear dose-dependent decrease in PROD activity,

because of suicide inhibition. And following exposure to nitrapyrin, we also see suicide inhibition here.

So this really explains the apparent inconsistency, why we didn't see the enzyme activity, while we did see the CYP2B10 gene and protein.

But it's important to note here that the PROD enzyme activity has been used in the past simply as a biomarker of CAR activation. And it doesn't play a role in this mode of action, but rather CAR activation causing hepatocellular proliferation, which is key event two.

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DR. LaROCCA: So moving on there with evaluation of hepatocellular proliferation in vivo, similar to what we saw in the key events number one, we see clear threshold based, dose-dependent increase in hepatocellular proliferation. Also, when we take nitrapyrin away, we see clear recovery of these effects, which is characteristic for this specific mode of action.

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DR. LaROCCA: So far to summarize, for the liver tumors, we see key event one, CAR activation, as evidenced by the CYP2B10 biomarker gene expression, associated liver weight and hypertrophy. And we see suicide inhibition of PROD, which helps evaluate a previously identified uncertainty by the CARC.

We also see clear evidence of key event two, hepatocellular proliferation.

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DR. LaROCCA: So while in the past we -- so sorry, we wanted to go on and exclude alternative modes of action, because while we have evidence to say that this is the evidence that we have to support this, we need to be able to really definitively exclude alternatives. So we did this by asking the question, is CAR necessary for nitrapyrin mediated liver effects, specifically proliferation, because proliferation is what's going to be causing these tumors. And so that's why we did the CAR knockout mouse study.

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DR. LaROCCA: And in this study, we analyze the same endpoints, the CYP2B10, the liver weight increases, and proliferation.

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DR. LaROCCA: And what was already shown to you is we do have a robust increase of CYP2B10. I believe that there was also other data shown earlier showing some spill-over effects into CYP1A1. I don't have the data shown here, but I'd like to just take the opportunity to point out that with the CYP1A1 increase, which was observed in the CAR knockout mice, this was around a

hundred fold, which is a lot less robust of a response that you would expect to see with prototypical activator, which instead of in the hundreds, it would be in the thousands.

So we think what's going on here is just a spill-over effect, because it's an artificial system and CAR is not there.

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DR. LaROCCA: But what's important here is with this mode of action, is key event 2, hepatocellular proliferation, and this is what is necessary to cause these tumors. And similar to what we saw in our mode of action study, in our wild-type mice we see a clear increase in hepatocellular proliferation, which is absent in our CAR knock-out mice.

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DR. LaROCCA: We also evaluated alternatives modes of action for coherence and plausibility using Bradford Hill criteria. And I'm not going to go into all of this data now. It's in the human relevance framework, but we did evaluate these and due to a lack of coherence or plausibility these could all be effectively ruled out.

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DR. LaROCCA: So for our conclusions, can exclude alternative modes of action? Yes, because CAR is

necessary for the nitrapyrin-induced hepatocellular proliferation, and alternative modes of action can be excluded due to a lack of plausibility or coherence.

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DR. LaROCCA: So we may have stopped there in the past, given that CAR activation can be considered to be not relevant for human health risk assessment due to qualitative differences, but we didn't think that that was good enough. So we took that a step further and thought we need to generate data specifically for nitrapyrin because we want to be sure.

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DR. LaROCCA: So how do we do that? How do we figure out if nitrapyrin mediated CAR activation is relevant to humans?

And one option that we could have used is those humanized mouse models. But the problem there is, you know, as was discussed with the phenobarbital, while there are quantitative differences in response, it's a human gene and a mouse, which has its limitations. So we chose not to go with that route.

So instead we decided to use primary mouse and human hepatocytes. And we wanted to use fresh, because we thought that there might be issues with cryopreserved. And by using fresh hepatocytes, this does require donors.

It has its limitations, but we did this this year and this is the data that we have.

So similar to what we saw in in vivo in mouse primary hepatocytes, following exposure to nitrapyrin, which was limited to cytotoxicity at 10 micromolar, we see a clear dose-dependent in proliferation.

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DR. LaROCCA: When human hepatocytes were exposed to the same or even 10-fold times higher, there's no change in proliferation, which demonstrates that nitrapyrin does not increase hepatocellular proliferation in humans.

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DR. LaROCCA: So to summarize, what we see in mice with key event one, CAR activation. And we see this with clear CYP2B10 gene expression increases; followed by key event 2, an increase in hepatocellular proliferation, which leads to tumor formation. And with here, we have a clear point of departure of 75 milligrams per kilogram per day, which is characteristic for CAR mode of action but would not be characteristic for another mode of action such as genotoxicity.

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DR. LaROCCA: These effects are effectively absent in the CAR knockout mice, so you would expect that

no tumors would be formed in these mice following a two-year chronic exposure. And while primarily mouse hepatocytes have a clear dose dependent increase in proliferation. This effect is absent in humans.

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DR. LaROCCA: So in conclusion, due to this mode of action and the data that we have, this concludes that these tumors not relevant for human health risk assessment. And the last CARC review in 2012, they did identify that the data were not sufficient to ascribe this mode of action, specifically for the PROD activity and the burst of mitotic activity. So we spent the last three years addressing these concerns, so we know that PROD, it's because of suicide inhibition, and the burst of mitotic activity we addressed by incorporating a four-day time point. We also went above and beyond this and did the CAR knockout study and the primary hepatocyte site study.

So while it was last reclassified as suggestive evidence, these additional studies can help support for other reclassification to not likely. And again, this was incorporated in a human relevance framework, which was recently submitted to the EPA and it's currently under review.

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DR. LaROCCA: So moving on to the histiocytic sarcomas in the epididymis, again these were only observed in the two highest doses in the mouse cancer bioassay in 1997. And they were originally misclassified as undifferentiated sarcomas, but the scientific advisory group reclassified these as histiocytic sarcomas, which were identical to the tumors observed in the 1990 study controls.

A pathology working group was also convened after that to clarify. And they confirmed that these are histiocytic sarcomas and they also identified additional tumor in the 1990 control mice.

So while when you look at the study, in and of itself, it may appear that there is a treatment related increase in these tumors. There is a low incidence in controls, particularly when you take into account the historical control range, as well as in the fact that the first mouse cancer bioassay, the incidence in unexposed controls was six percent, which exceeds that historical control range.

So taking this into account, you can combine these two studies to get a real dose response for nitrapyrin. And as you can see, for instance, in the unexposed controls in the 1990 studies, three mice had histiocytic sarcomas in the epididymis compared with two

in the 125 and four in the 250 milligrams per kilogram per day treatment groups.

So taking this into account, the pathology working group concluded that due to the incidence in distribution, these are spontaneous and they're unrelated to treatment to nitrapyrin --

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DR. LaROCCA: -- which is in conjunction with our conclusions as well as with the U.S. EPA CARC decision in 2012.

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DR. LaROCCA: Moving on to the harderian gland tumors, again these are harderian gland is found in the orbital sinus of mice, but is not present in humans. And histio -- and harderian gland tumors were found at the two highest dose groups.

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DR. LaROCCA: But similar to what was seen with the histiocytic sarcomas, there was an unusually low incidence in the controls with only two percent having this tumor. And with this historical control range, from the same lab within the same three years, the range is six to 10. So taking this into account, it's appropriate to combine these two studies together.

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DR. LaROCCA: And here, you can see due to the incidence in distribution of these tumors and the lack of a dose response between 125 and 250, milligrams per kilogram, these tumors are really representing a numerical imbalance and they're not treatment related. And this was the conclusion made by the independent group of pathologists from the scientific advisory group.

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DR. LaROCCA: This is also in conjunction with our conclusions, as well as the CARC in 2005, as well as in 2012 that these are not treatment related.

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DR. LaROCCA: So finally, concluding, moving on from the mice and back into the rat with kidney tumors.

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DR. LaROCCA: These were observed in males only at an incidence of six percent. And this phase occur due to the alpha-2u-globulin nephropathy which is a mechanism nephropathy, which is a mechanism considered to be not relevant to humans. And the reason they're not relevant to humans is because alpha-2u protein is absent in humans. It's also relatively absent in female rats, which is why you see tumors only in males and you don't see them in the females.

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DR. LaROCCA: So keeping this in mind, there are several criteria that you should meet for the alpha-2u-globulin mechanism. And as far as the EPA criteria, we have data to support that we meet these very clearly, including the number and size of hyaline droplets. We have evidence to clearly show that the protein is alpha-2u, and we do have the pathologic sequence of lesions going from 12 to 24 months.

I'd also like to point out that this evidence was only observed in nitrapyrin treated male rats, and was absent in female rats. The only evidence in female rats for any kind of renal issues was an increase in the proteinaceous casts, so you got tubule dilation. But this really shows an exacerbation of something that already occurs in these animals

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DR. LaROCCA: So in conclusion for the kidney tumors, these do meet several of the criteria for establishing this mechanism, which is considered to be not relevant for human health risk assessment, which was reiterated in the 1992 CPRC decision by the U.S. EPA.

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DR. LaROCCA: So just to sum everything up beginning with the genotoxicity. The interpretation is that nitrapyrin is not genotoxic. And while there is a

single weak positive response in an Ames test, we do have higher tiered in vitro testing, as well as two separate in vivo studies. There was an expert review by Dr. Zeiger, who was the author of the positive NTP report, and he determined that there is no concern for mutagenic mode of action. And this is in agreement with the U.S. EPA decision.

Regarding the forestomach neoplasms, there are qualitative differences between mice and men, given the fact that mice have a forestomach and we do not. We also have evidence to show that targeting other tissues, such as the esophagus or oral cavity, given the differences between a storage organ and not, it's unlikely that you'd see any kind of irritation in these organs. The histiocytic sarcomas and harderian gland tumors are both spurious and not treatment related, which was validated by the U.S. EPA decisions in 2005 and 2012.

The kidney tumors occur in male rats as due to alph-2u-globulin nephropathy, which is not relevant for risk assessment. And then finally, with the liver tumors, we have data to support that it is CAR activation, which is the mode of action. We also have nitrapyrin specific data for the primary mouse and human hepatocytes, which demonstrate that the particular key event, number two, which is hepatocellular proliferation, would not occur in

humans, as it does occur in mice.

We also generated additional data to satisfy some of the uncertainties, including PROD, and have incorporated this in an updated human relevance framework, and submitted this to the EPA.

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DR. LaROCCA: So taking this into account, we'd like to support that nitrapyrin does not meet the criteria of scientifically testing, according to generally accepted principles to cause cancer in humans, which is consistent with the proposed delisting of nitrapyrin.

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DR. LaROCCA: With that, I'd like to thank all of my colleagues at Dow AgroSciences, as well as in TERC. And I'd also thank you. And I saved five minutes, so I did my best.

I'll take any questions.

CHAIRPERSON MACK: Okay good. David.

COMMITTEE MEMBER EASTMOND: Nice presentation. I mean, I think it's impressive what you've done on sort of working sequentially through these and some of your work with CAR, and certainly alpha-2u-globulin. The one that I kind of get hung up on is the harderian gland tumors, because it seems to me that the incidence of tumors seen on the highest doses is essentially double the historical

1 controls, even the range -- you know, they're double the
2 range of the historical controls.
3 And the -- you know, for me, that strikes --
4 looks at a sort of treatment related effect. Now,
5 obviously it's not a clean dose response curve, but
6 they're clearly elevated, and one is even higher than the
7 other. Any additional thoughts as to why you think that's
8 spurious or --
9 DR. LaROCCA: Sure. So what I showed you today
10 was the female data. And there's no evidence to suggest
11 that females versus males would be more or less
12 susceptible to forming harderian gland tumors. And the
13 historical control range for the females falls into kind
14 of the overall combined for males and females. For some
15 reason, the males it's between six and 18 percent within
16 that three-year timespan in the lab. In females, it was
17 six to 10 percent. So it does fall slightly out of the
18 historical control range. But as far as -- and then
19 there's no declared dose response.
20 COMMITTEE MEMBER EASTMOND: That's when you bring
21 the male historical control in, if you focus on the female
22 historical controls. And in the document, I'm not sure,
23 they use the NTP range, which is in -- you know, it's
24 smaller up to 10 -- zero to 10. And then they further to
25 the Charles River company study. And I'm assuming that's

the origin of the animals was from Charles River, or did they just pick a -- just as a reference.

DR. LaROCCA: Actually, I don't know, off the top of my head, what the origin of the animals was.

COMMITTEE MEMBER EASTMOND: Okay. All right.

DR. LaROCCA: What I do know is that for the harderian gland tumors, we do have in our response the historical control range, which was from our lab within that three-year timespan, because we thought that that would be fitting most appropriate range for the historical controls. Whereas, originally, we did have the NTP and a range back from the 80s.

CHAIRPERSON MACK: Joe.

COMMITTEE MEMBER LANDOLPH: Yeah. Two questions. One was I was looking at the other data from the hazard identification document and we've listed pyridine before, which had no genotoxicity but one, two, three, four, five tumor endpoints positive. And the nitrapyrin has a little bit of genotoxicity and one, two, three, four, five, six, seven tumor endpoints.

Now, certainly, I think your presentation was very elegant, but it's difficult for me to throw away seven tumor endpoints. And I'm forced into a position where if we don't list nitrapyrin, then I think the Committee would have to reconsider pyridine, which I'm not

willing to do at this point. Do you have any thoughts about that?

DR. LaROCCA: Yeah, I think that's a really excellent point. And as far as pyridine is concerned and the structure activity relationships, as we all know, while we can have structures that are very similar, minor changes in the structure can drastically impact the toxicity, which is why we should rely on the data that we have for these molecules.

And I don't know the -- I don't know specifically every kind of endpoint for a pyridine, but what I do know is, for instance, our data for the kidney tumors is different for the lack of the alpha-2u mechanism. For a pyridine, I believe it was due to the fact that tumors were observed in the kidneys where they didn't have evidence for the alpha-2u protein, whereas we do have that for nitrapyrin, so that's a marked difference.

We also have all of the higher tiered genetox assays. We have the HGPRT, the unscheduled DNA synthesis test and we have the two in vivo assays, all of which were clear negatives. I also believe that it was brought up earlier the metabolite nitrapyrin, 6-CPA, may also have a concern. And you don't have the data in front of you, but what we have done in the past is we have done genetox assays on 6-CPA and those were clearly negative.

We also have a two-year cancer bioassay on 6-CPA. And no tumors were formed at any site. And all of the evidence actually suggests that 6-CPA is just less toxic across the Board. So we do have more data for that molecule. So I hope that helps answer your question.

COMMITTEE MEMBER LANDOLPH: Thank you for your effort. I'm not sure it does, but I think you did a valiant effort trying to answer that question.

CHAIRPERSON MACK: Jason, do you have any questions?

COMMITTEE MEMBER BUSH: Yeah. Just some minor things. So the presentation that you just gave was informative. Thank you. The document that we received, you're lead the author on it from the 2005 study, study ID 150067. I would suggest that if you're going to include data -- I'm looking at Figure 2 specifically, the phenobarbital, the PROD enzymatic activity that you did or CYP enzyme induction suicide inhibition. The figure that you show here is a lot better than what you've got in this.

So we only have this information in front of us. And, you know, I think in the future if you're trying to mitigate any points that at least the data is consistent. It looks better in here than in this document. So this is the one we have access to at the time we're making our

evaluation. So best keep things consistent and then we can review, you know, the correct data, I suppose.

You know, the error bars are a little bit extra different. You've got some extra things in there, and vice versa. So that would be my only comment about the information that we have for it.

DR. LaROCCA: I think that maybe the difference in those groups -- I can't remember off the top of my head, but it was the phenobarbital and the curcumin that's the same, and everything with the nitrapyrin with error bars is the same. But I think in the presentation at one point, we had another dose in the middle, but we didn't have any replicates, so that's why there was no error bars for that one. And because there was no replicates, we didn't think it was as conclusive as the other ones.

CHAIRPERSON MACK: Joe.

COMMITTEE MEMBER LANDOLPH: Is it possible that the weak mutagenicity of nitrapyrin, in combination with CAR or something like that, is giving you mixed mechanism for this compound for the mode of action?

DR. LaROCCA: I don't believe so, so my short answer is no. But the reason that I say that is because with the liver tumor mode of action studies, we meet several of the criteria using well established practices, such as Bradford Hill criteria for establishing CAR as the

mode of action. And along with that, we have the battery of additional genotoxicity assays, which suggests that nitrapyrin is not genotoxic.

In the sense that if you would see a mixed effect with genotoxicity, there wouldn't be such a clear point of departure as what we have say with the liver tumor effects.

COMMITTEE MEMBER LANDOLPH: The reason I ask that question is just last week I taught graduate students a course in carcinogenesis. And one of the lectures was on furan and furfural, which were negative for mutagenicity, but when you take the tumors out, it was published the tumors have activating mutations in ras genes, so you can be fooled by lack of -- apparent lack of mutagenicity data. Thank you.

COMMITTEE MEMBER DAIRKEE: I have a question.

CHAIRPERSON MACK: Shanaz.

COMMITTEE MEMBER DAIRKEE: You make this point about comparing mouse hepatocytes and human hepatocytes and not seeing proliferation in the primary cultures of the humans. But you only have an N of 2 in the human and humans are not inbred mice, so you don't expect to see that until you do a good enough N. So I think that data is still weak.

DR. LaROCCA: Yeah. No, you bring up a good

point. And the limitations of using this assay, again we could have done a higher N's had we cryopreserves, but we thought that using the primary fresh human hepatocytes was the best way to go. But to be perfectly honest with you, we need human donors to pass away in order to get these. So the donors are few and far between. But I do accept that that's a limitation, but we thought that this was the best assay that we had available. And still I think it's probably the best assay that we have.

CHAIRPERSON MACK: Luoping.

COMMITTEE MEMBER ZHANG: (Shakes head.)

CHAIRPERSON MACK: Nothing. Okay.

Has nitrapyrin been clearly shown through scientifically valid testing, according to generally accepted principles to cause cancer? Everybody who wishes to say yes to that proposition, raise their hand.

(Hands raised.)

CHAIRPERSON MACK: Looks like we have five positives.

Everybody who says no raise their hand.

(Hand raised.)

CHAIRPERSON MACK: Everybody who wishes to abstain.

(Hand raised.)

CHAIRPERSON MACK: One. So 5 to 1 to 1.

You convinced me.

(Laughter.)

CHAIRPERSON MACK: For better or worse, although you didn't convince much more knowledgeable people.

(Laughter.)

CHAIRPERSON MACK: Now we go to other issues. Staff updates who's going to do that?

Michelle. There she is. Hiding.

(Thereupon an overhead presentation was presented as follows.)

MS. ROBINSON: Over here. You can hear me now, right?

Okay. So for those of you that couldn't see me before, my name is Michelle. I'm new to the Prop 65 implementation program. I was told that it was my option to read off all the chemicals here. And I figure that I would only do that if I could sing them, but fortunately for you I'm not that creative, so it's there for you to read if you'd like.

You'll see the last row here, it says pending for the date. These are the administratively added chemicals since December 2014, since the time you last met, but that last row says pending. Carol will talk about that when she comes up in a little while.

Are there any questions on this slide?

COMMITTEE MEMBER ZHANG: Could you speak a little bit louder? I can't hear you.

MS. ROBINSON: Here you go. I just didn't want to scream into the microphone, but I guess we have to, huh? Okay.

Okay. Still learning. All right.

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MS. ROBINSON: All right. Now the next slide is the chemicals under consideration. There are six columns -- or six rows here.

Any questions on these?

Okay.

And then we have

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CHAIRPERSON MACK: Go back one just a minute.

MS. ROBINSON: Oh, you want to go back. Okay.

You got it all?

Okay. Any other questions?

Okay. Two more rows.

Everybody have all these?

Any questions on these?

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MS. ROBINSON: Moving on. And this one here we have the list of the proposed safe harbor levels. There's just one. Any questions?

Okay. And I think that's the last slide. Yep. Short, sweet, to the point.

CHAIRPERSON MACK: Thank you.

What's next, boss?

ACTING DIRECTOR ZEISE: Proposition 65 litigation.

CHAIRPERSON MACK: There she is.

CHIEF COUNSEL MONAHAN-CUMMINGS: Hi. This is Carol Monahan-Cummings. I wanted to give you a very brief update on our current litigation under Prop 65. Our office has set an all-time record for the number of cases that are currently pending. We have seven, one of which actually isn't a Prop 65 case. It has to do with a challenge to our public health goal for perchlorate. But with that exception, all the other cases that we have pending right now are related to Proposition 65. Some of them you have heard about before.

We have three cases currently pending that were filed by Syngenta Crop Protection. The first one is a challenge to the no significant risk level for chlorothalonil which is a listed carcinogen under Prop 65. Currently, that case is stayed pending a request by Syngenta for a safe use determination for the chemical, as it is used on 175 different food products.

So we're considering the -- that request at the

moment. And so the case has been stayed. The second one has to do with the listing of the triazine -- the set of triazine chemicals that you saw on an earlier slide as developmental or reproductive toxicants. The listing in that case has been made, but has -- the effective date has been delayed, because of the challenge to the listing by Syngenta.

And currently, there's a hearing scheduled for next week on November the 13th on the merits of that -- that case to find out whether or not the trial court will overturn the listing. In the event that that occurs, most likely the case will be appealed. And so either way, whether the court upholds the listing or does not uphold the listing, I would imagine the case will go up on appeal.

And because of that, there is a -- we had mentioned that there were some -- or maybe we didn't for this group, but there's safe harbor levels for the triazine chemicals that are pending, also depending on whether or not the chemicals are eventually listed, if that make sense. There's no reason to have a safe harbor level if the chemicals aren't listed.

The third case filed by Syngenta is related to the triazine case, and that's a challenge to a Public Records Act request that they made. And we produced

records, but not to their satisfaction, so that case is trailing the other two.

We also have a case filed against OEHHA by Mateel Environmental Justice, and they have challenged the current safe harbor level for lead. Lead was listed many years ago under Prop 65, and we have had a safe harbor level in place for nearly -- well, at least 25 years. And so there's -- there was a challenge to that that is now pending in the trial court. There's a hearing in that case on December the 17th, and we should have a decision probably early next year in that case, at least at the trial level, about whether or not the existing MADL is legal, as it was adopted back in 1989 or so.

Related to that, we had a petition to relook at the safe harbor level for lead, which we are actually in the process of doing. We just had a pre-regulatory hearing on a proposal for new MADLs, actually a number of them, for intermittent exposures to that compound.

I already mentioned the case that was filed by the California Manufacturers and Technology Association - is that correct - challenging our public health goal for perchlorate. We have two cases that are currently on appeal. The first one was filed by the American Chemistry Council challenging the original listing of BPA, bisphenol A, as a developmental toxicant. And that case is up on

appeal. There's a briefing schedule now, and we expect that there will be a decision by the court of appeal sometime next year -- probably early next year.

The second case that's on appeal is more related to the work that you all are doing, and that is the -- a challenge by the American Chemistry Council to this Committee's listing of the chemical DINP. As you may recall, we were successful at the trial level in defending your listing of that chemical, but the case was appealed. And again, there is a briefing schedule now in that case, and we expect a decision sometime probably towards the middle of next year.

Any questions?

COMMITTEE MEMBER LANDOLPH: Do we have to hold on to any DINP literature we have received?

CHIEF COUNSEL MONAHAN-CUMMINGS: Yes, I think that the litigation hold is still in place in that case. Just in an abundance of caution in the event that the listing is overturned, we may need to go back around again. So I would appreciate if you'd maintain those records.

COMMITTEE MEMBER LANDOLPH: And if we lost it, you can provide it again, I guess?

CHIEF COUNSEL MONAHAN-CUMMINGS: Well, if you lost it, we probably have it, so -- but just don't

intentionally lose anything.

Any questions?

Thank you.

CHAIRPERSON MACK: Our current chairman will summarize.

(Laughter.)

ACTING DIRECTOR ZEISE: Okay. Thank you. So I'll summarize the Committee's activities -- decisions today. So the Committee considered diaminotoluenes mixed, as well as isomers of diaminotoluenes with the exception of 2,4-diaminotoluene. The Committee voted unanimously that diaminotoluene mixed was not clearly shown through scientifically valid testing, according to generally accepted principles to cause cancer. So diaminotoluenes will be removed from the Prop -- diaminotoluene mixed will be removed from the Proposition 65 list.

The Committee also considered 2,3, 3,4 and 3,5-diaminotoluene. And in their vote, they unanimously voted that it was not clearly shown, and so those compounds won't be added on the list.

The Committee voted 3 yes, 3 no, and 1 abstention that 2,5-diaminotoluene had been clearly shown. And for 2,6 the vote was 3 yes, 4 no, 1 abstention, so neither of those isomers will be added to the list either.

For nitrapyrin, the Committee voted 5 yes, 1 no,

and 1 abstention that nitrapyrin had been clearly shown through scientifically valid testing, according to generally accepted principles to cause cancer. So nitrapyrin will remain on the list.

So that sums up the Committee's activities.

Any questions or...

CHAIRPERSON MACK: Any further questions or issues?

ACTING DIRECTOR ZEISE: Okay. So now on to -- did you want to say something more?

CHAIRPERSON MACK: No, I don't want to say anything more.

(Laughter.)

ACTING DIRECTOR ZEISE: Okay. I'll keep going then. So I did want to thank the Committee for taking time out of their busy schedules and donating time to the State of California and your expertise for considering these compounds. They were -- the scientific evidence was complex. And I did want to acknowledge -- Martha reminded me this morning that the Committee hasn't met since Dr. George Alexeeff passed away. He was our much beloved director. And I know that he would be -- he loved science. And I know he'd be very pleased by the Committee's careful thinking and all the work that you put in. So I did want to acknowledge that as well.

I'd also like to thank the members of the public here in the room, the presenters, and those joining us on the web for your participation in our Proposition 65 activities, and for coming to this meeting. And I'd like to thank our RCHAB staff for the tremendous amount of work that they put into these presentations and to the very well done documents, to our legal staff for all the support, and to our implementation office. So thank you all.

CHAIRPERSON MACK: So thank you all, and good night.

(Thereupon the Carcinogen Identification Committee adjourned at 3:15 p.m.)

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

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

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

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

IN WITNESS WHEREOF, I have hereunto set my hand this 16th day of November, 2015.

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