MEETING

STATE OF CALIFORNIA

ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

PROPOSITION 65

CARCINOGEN IDENTIFICATION COMMITTEE

CALEPA HEADQUARTERS BUILDING

1001 I STREET

SIERRA HEARING ROOM

SACRAMENTO, CALIFORNIA

THURSDAY, NOVEMBER 1, 2018

10:06 A.M.

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A P P E A R A N C E S

COMMITTEE MEMBERS:

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

Jason Bush, Ph.D.

Shanaz Dairkee, Ph.D.

David A. Eastmond, Ph.D.

Thomas McDonald, Ph.D., M.P.H.

Michelle La Merrill, Ph.D.

Joseph Landolph, Ph.D.

Peggy Reynolds, Ph.D.

Mariana Stern, Ph.D.

Luoping Zhang, Ph.D.

STAFF:

Dr. Lauren Zeise, Director

Ms. Carol Monahan Cummings, Chief Counsel

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

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

Mr. Julian Leichty, Proposition 65 Implementation Program

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

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

Dr. Feng Tsai, Reproductive and Cancer Hazard Assessment Branch, Cancer Toxicology and Epidemiology Section

I N D E X

P R O C E E D I N G S

DIRECTOR ZEISE: So good morning, everyone, and welcome to the Carcinogen Identification Committee meeting. Dr. Mack, our Chairperson, his plane was delayed, so Dr. Eastmond is going to be acting as Chair until he arrives, which should be in 15 or 20 minutes. So we'll get started.

We have two main agenda items. First, the consideration of gentian violet, and then the consideration of n-nitrosohexamethyleneimine. So the consideration by the Committee of those chemicals as known to the State the cause cancer.

The meeting is being transcribed and webcast, so if everyone could please speak directly into their mics. And then I just want to take a few minutes to announce some logistics. The drinking fountains are -- and the restrooms are out the black door, turn left, walk to the end of hall.

In the event of a need to evacuate the room, please leave by the lighted exit doors, and then take the steps down, and out -- walk outside so -- to your right, take the steps down, and walk outside, and across the street. And we'll relocate in the park across the street.

So we're going to be taking breaks during the meeting for the court report, typically five minute

breaks.

And now, we'll introduce the Panel.

So I'll go along this direction. Dr. Luoping Zhang from the University of California, Berkeley, School of Public Health. Then Dr. Peggy Reynolds, Cancer Prevention Institute of California, and Stanford University School of medicine. Then our new member, Dr. Mariana Stern, University of Southern California, Keck School of Medicine. Then Dr. Joe Landolph, University of Southern California, retired. And then Dr. David Eastmond, UC Riverside, Molecular Cell and Systems Biology Department. And then Dr. Michelle La Merrill, UC Davis and Lawrence Berkeley National Laboratory. Dr. Thomas McDonald, Clorox Company, Global Stewardship. Dr. Shanaz Dairkee, California Pacific Medical Center. And Dr. Jason Bush, California State University, Fresno, Biology Department. So welcome, Panel.

And now I'll introduce the OEHHA staff. Carol Monahan Cummings our Chief Counsel. Martha Sandy, Chief of the Reproductive and Cancer Hazard Assessment Branch. And then making presentations today next to Martha is Meng Sun -- Dr. Meng Sun. Next to her Dr. Karen Ricker. Next to her Dr. Feng Tsai. Then Dr. Jennifer Hsieh. And Dr. Gwendolyn Osborne. So that's our RCHAB staff. I'd also like to introduce Sam Delson, who's our Deputy Director

for External Affairs.

And now I'll turn to our Proposition 65 Implementation Program staff. Esther Barajas-Ochoa in the corner there, and Julian Leichty.

So, welcome. So before we get started and I turn over the meeting to Dr. Eastmond, I'd like to swear in our two new members, Dr. Mariana Stern and Dr. Michelle La Merrill. So if you would please stand and come in this direction.

A mic.

Hello. Is it on?

COMMITTEE MEMBER EASTMOND: You're on.

DIRECTOR ZEISE: I'm on. Okay. Great.

So if you could please raise your right hand and state your name, I --

COMMITTEE MEMBERS: I --

DIRECTOR ZEISE: -- do solemnly swear --

COMMITTEE MEMBERS: -- do solemnly swear --

DIRECTOR ZEISE: -- that I will support and defend --

COMMITTEE MEMBERS: -- that I will support and defend --

DIRECTOR ZEISE: -- the Constitution of the United States --

COMMITTEE MEMBERS: The Constitution of the

United States --

DIRECTOR ZEISE: -- and the constitution of the State of California --

COMMITTEE MEMBERS: -- and the Constitution of the State of California --

DIRECTOR ZEISE: -- against all enemies foreign and domestic --

COMMITTEE MEMBERS: -- against all enemies foreign and domestic --

DIRECTOR ZEISE: -- and that I will bear truth faith and allegiance --

COMMITTEE MEMBERS: -- and that I will bear true faith and allegiance --

DR. ZEISE: -- to the Constitution of the United States --

COMMITTEE MEMBERS: -- to the Constitution of the United States --

DIRECTOR ZEISE: -- and the Constitution of the State of California --

COMMITTEE MEMBERS: -- and the Constitution of the State of California --

DIRECTOR ZEISE: --- that I take this obligation freely without any mental reservation --

COMMITTEE MEMBERS: -- that I take this obligation freely without mental reservation --

DIRECTOR ZEISE: -- or purpose of evasion --
COMMITTEE MEMBERS: -- or purpose of evasion --
DIRECTOR ZEISE: -- and that I will well and faithfully discharge the duties upon which I am about to enter --
COMMITTEE MEMBERS: Maybe do it again.
(Laughter.)
DIRECTOR ZEISE: Okay. That I will well and faithfully discharge the duties upon which I am about to enter.
COMMITTEE MEMBERS: That I will well and faithfully discharge the duties upon which I am about to enter.
DIRECTOR ZEISE: So welcome to the Panel.
(Applause.)
DIRECTOR ZEISE: Okay. Now, before we get into the meat of the meeting, Carol is going to make some introductory comments.
CHIEF COUNSEL MONAHAN CUMMINGS: Good morning. Most of you have heard these before, some of them not. But I try to remind the Committee of a number of things at each meeting, since you only meet once a year. First, I would like to remind you that the listing criteria that's been adopted by this Committee is in your binders under criteria, I believe.

That criteria was adopted by the Committee to help you make decisions about potential listing of chemicals. Your decision should be based on that criteria, not on consideration of the future impact of a listing, such as whether or not warnings would be required for a particular exposure.

Your charge is to determine whether the chemicals that are being presented are clearly shown through scientifically valid testing, according to generally accepted principles to cause cancer. The standard is a scientific judgment call. It's not a legal standard of proof.

Your Committee can decide to list a chemical based on -- only on animal evidence. The chemical need not have been shown to be a human carcinogen, and whether or not there are human exposures to the chemical, or whether or not current human exposures to the chemical are sufficiently high enough to cause cancer.

The members of this Committee were appointed by the Governor because of your scientific expertise and are considered the State's qualified experts on carcinogenicity of chemicals. There's no need to feel compelled to go outside that charge.

In the event you feel you have insufficient information or need more time to think or discuss the

issues in front of the Committee, there's no requirement that you make a decision today. You could defer your decision to another meeting and give staff suggestions on the information you feel like you need, and we're happy to get that information if it's available and present it at a future meeting.

Feel free also to ask clarifying questions of me or the other OEHHA staff during the meeting. If we don't know the answer to your question, we'll do our best to find it and report it back to you.

Any questions this morning?

CHIEF COUNSEL MONAHAN CUMMINGS: Okay. Thank you.

DIRECTOR ZEISE: Thank you, Carol. And now I'll turn the meeting over to Dr. Eastmond.

COMMITTEE MEMBER EASTMOND: Well, thank you. As Lauren mentioned I'll be filling for Tom Mack until he arrives, hopefully shortly. And I'd just like personally to express my welcome to our new Committee members. Glad to have you involved, and hopefully it will be an interesting and valuable experience for all us.

It's my understanding that we do not have any public comments. Do we have any at this point?

Just, if there are people in the public who would like to make comments, per our usual sort of model, each

speaker in the public has five minutes to speak. And there are blue cards available in the back table. If you'd like to make public comments, please fill one out and give them to either Esther or Julian.

But, at this point, I don't think we have any.

The -- as typical, we will have staff presentations on each of the chemicals, and then -- so we'll start with gentian violet, I believe. And Dr. Martha Sandy will introduce the OEHHA staff and the chemical.

DR. SANDY: Thank you, Dr. Eastmond.

(Thereupon an overhead presentation was Presented as follows.)

DR. SANDY: So gentian violet was brought to your Committee back in 2010 for prioritization. And so that's the origin of how it's coming to you now. It was selected for development of this document before you, and for your consideration today. And we're going to be hearing from a few of the authors of the document. We'll lead off with -- it will be Dr. Meng Sun and Dr. Ricker that will be presenting on this.

Thank you.

DR. RICKER: Good morning, everyone. We are here today to present a summary of the evidence on the carcinogenicity of gentian violet.

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DR. RICKER: Here is a brief overview of today's presentation. We will start with background information, including identity of gentian violet, use and exposure, then reviews by other agencies. Next, we will talk about studies in humans, followed by a summary of the findings from animal cancer bioassays.

Lastly, we will present mechanistic and other relevant data.

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DR. RICKER: Gentian violet shown here on the right is also known as crystal violet and refers to hexamethylpararosaniline chloride, a cationic triphenylmethane dye derived from aniline.

Gentian violet produces a vibrant purple color, and has longstanding use as a biological and histological dye. It is a key stain in the Gram method for categorizing bacteria, and is also used as a nuclear stain for eukaryotic cells. Commercial uses of gentian violet include the coloration of paper, textiles, and elastic fibers, and the production of inks and toners.

Gentian violet is known to have antimicro -- antimicrobial properties. In the U.S., gentian violet is available as an antibacterial foam, and as one to two percent solutions intended for topical first aid uses.

In the context of breast feeding, recommendations for the use of gentian violet to treat infant oral thrush and thrush of the nipple can be found on many websites, including those of medical practitioners.

Other uses of gentian violet discussed on the internet include its use in making do-it-yourself purple hair dyes.

Gentian violet is not permitted in animal feed, including fish feed, nor is it permitted as a veterinary drug in food animals in the U.S. The U.S. Food and Drug Administration regularly monitors domestic and imported seafood for gentian violet residues, and over the years has issued several import alerts for seafood containing gentian violet residues from a number of countries.

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DR. RICKER: Gentian violet has been of interest to several regulatory agencies. FDA considers gentian violet, "a suspected carcinogen, a probable mutagen, and a potent clastogen". NTP referred to gentian violet as a carcinogenic dye in its report on two structurally related compounds.

The Joint FAO/WHO Expert Committee on Food Additives has concluded that it is inappropriate to set an acceptable daily intake for gentian violet, because it is genotoxic and carcinogenic.

The Australian Pesticides and Veterinary Medicines authority found that gentian violet demonstrated carcinogenic/tumorigenic effects in mice, and that it is a mutagen and clastogen, and canceled the registrations and approvals of products containing gentian violet.

With that, I'm handing the presentation over to Dr. Sun.

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DR. SUN: Available evidence for the carcinogenicity of gentian violet in humans is sparse. We identified a hospital-based retrospective study conducted in Brazil in 1989. 4,765 patients were interviewed and asked if they recalled ever receiving gentian violet-treated blood. Of the 37 patients who answered yes, 26 had either benign or malignant neoplastic lesions.

There are several limitations to this study, including lack of information on the specific site or type of cancer observed, lack of information on any comparison groups, selection bias, because the patients were from a hospital that was affiliated with combating cancer and confounding factors, such as higher iron levels immunosuppression that may occur in recipients of blood transfusions.

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DR. SUN: Now, we will turn to the available

evidence in animals. There are four animal cancer bioassays of gentian violate, one each in male rats, female rats, male mice, and female mice. These were all feed studies. In the male and female rat studies, exposures began in utero and continued during lactation via dosing of the dams, and then continued with direct dosing of the pups after weaning through 24 months. The studies in rats included 12- and 18-month interim sacrifices.

In the male and female mouse studies, exposures began post-weaning at four to five weeks of age for up to 24 months. These mouse studies also included 12- and 18-month interim sacrifices.

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DR. SUN: Here are the tumor findings in male F344 rats. Tumor were seen in multiple sites in male rats exposed in utero, during lactation, and via feed post-weaning for up to 24 months. No tumors were observed in any site in the animals sacrificed at 12 months. The table shows tumors observed at the 18-month interim sacrifice and in the animals on test for up to 24 months.

A significant increase in hepatocellular adenoma was observed in the highest dose group by pairwise comparison with controls with a significant dose-related trend. Thyroid gland follicular cell adenocarcinomas were

observed in the low- and high-dose groups with a dose-related trend. Follicular cell adenomas and adenocarcinomas combined were increased in the high-dose group with a dose-related trend. The incidences of mesotheliomas of testis and epididymis which were reported only as percentages were increased in the mid- and high-dose groups in both the 18-month sacrifice groups and the animals on test for up to 24 months.

I'll just continue.

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DR. SUN: The female rat study had the same study design and exposure regimen as the male rat study. No tumors were observed at any site in the animals sacrificed at 12 months. Data are presented from the 18-month interim sacrifices and from animals exposed for up to 24 months.

Increases in thyroid gland follicular cell adenoma[SIC], and adenoma or adenocarcinoma combined were observed in the mid- and high-dose groups with dose-related trends. These tumors are rare in untreated female F344 rats.

In the 18-month interim sacrifice groups, the incidence of mononuclear cell leukemia was significantly increased in the highest dose group, with a dose-related trend. Although no treatment-related increase in this

leukemia was apparent in animals exposed for up to 24 months, it appears that gentian violet reduced the latency of the leukemia. NCTR concluded that dosing with gentian violet was significantly associated with an earlier onset and increased mortality due to leukemia.

The incidences of clitoral gland adenoma or adenocarcinoma combined, which were reported only as percentages, were increased in the mid- and high-dose groups.

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DR. SUN: This slide summarizes tumor findings in the male mouse study. Animals were exposed at four to five weeks of age for up to 24 months. Data presented from the 12- and 18-month sacrifices as well as from animals treated for up to 24 months.

Increases in hepatocellular adenomas were observed in the mid- and high-dose groups with a dose-related trend. Hepatocellular carcinomas were observed in the high-dose group with a dose-related trend. The reporting of the data by NCTR did not allow us to determine the combined incidence of hepatocellular adenomas and carcinomas.

Increases in Harderian gland adenomas were observed in the mid- and high-dose groups with a dose-related trend.

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DR. SUN: This is the first of two slides summarizing results from the female mouse study. Animals were exposed at four to five weeks of age for up to 24 months. Data are presented from the 12- and 18-month interim sacrifices and from the animals treated for up to 24 months.

Increases in hepatocellular adenoma and carcinoma were both observed in the mid- and high-dose groups with significant trends. The reporting did not allow us to determine the combined incidence. An increase of hepatocellular adenomas was also seen in the high-dose group with a dose-related trend at the 18-month interim sacrifice.

Increases in Harderian gland adenomas were observed in all three treated groups with a dose-related trend.

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DR. SUN: Also, in the female mouse study, significant increases in type A reticulum cell sarcomas were observed in the mid- and high-dose groups by pairwise comparisons, with a significant trend, in each of the following tissues: Bladder, ovaries, uterus, and vagina.

Type A reticulum cell sarcoma is an older term that is no longer used by tumor pathologists. The current

classification for this tumor type is likely to be histiocytic sarcoma. We note that this is different from what was proposed in the HID. Now, I will hand it over to Dr. Ricker.

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DR. RICKER: Thank you, Dr. Sun. We are moving on to other relevant data, beginning with pharmacokinetics and metabolism.

No in vivo human metabolism studies of gentian violet were identified. However, there are in vitro studies of gentian violet metabolism conducted with human intestinal microflora, as noted here.

With regard to animal studies, the pharmacokinetics and metabolism of gentian violet has been studied in several species in vivo, and in liver microsomal systems isolated from several species. In vitro studies of gentian violet metabolism have also been conducted with intestinal microflora isolated from rats and chickens.

Other metabolism studies include those with various fungi and bacteria, and studies in cell-free systems.

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DR. RICKER: Absorption studies of gentian violet in mammals are limited. They indicate rapid but

incomplete absorption by the oral route. In rats, absorption within two hours can be indirectly estimated to be less than 10 percent based on measures from urinary and biliary excretion experiments.

In rats and mice, gentian violet was rapidly distributed throughout the body with the highest levels occurring in kidney and liver. Gentian violet and metabolites accumulated in adipose tissue and reached a plateau at 24 hours; and fatty tissue also contained the highest concentration of reduced metabolites.

Bile duct cannulation studies conducted in female rats reported that 5.7 to 6.4 percent of the administered dose of gentian violet was excreted in the bile within 28 hours. Gentian violet is excreted primarily in the feces with some excretion also via urine.

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DR. RICKER: I will now walk you through the proposed metabolism of gentian violet based on information from in vivo and in vitro studies, as well as observations from cell-free experiments and biodegradation studies. Chemical names shown on this slide in bold indicate that a metabolite has been detected in mammalian systems.

Let's start with oxidative metabolism. During oxidative metabolism, gentian violet undergoes N-demethylation, i.e. the stepwise removal of methyl

groups from the parent molecule. The stepwise removal leads to the formation of penta-, tetra-, tri-, and dimethyl pararosaniline as shown here.

Each removal of a methyl group also leads to the formation of formaldehyde, a known carcinogen, shown here in red. Complete demethylation of gentian violet can yield a carcinogen pararosaniline, which is also known as C.I. Basic Red 9.

Pentamethyl-pararosaniline and two isomers of tetra-methyl-pararosaniline have been detected in mammalian systems. Further demethylation products of gentian violet have not been assessed in mammalian systems. However, the complete demethylation product C.I. Basic Red 9 has been detected in microbial metabolism studies.

The oxidation pathway may also involve the formation of a nitrogen-centered free radical, which has been detected in cell-free systems using horseradish peroxidase. This part of the figure in the HID was presented with an error, but the correct figure is showing here on this slide.

We are now moving to reductive metabolism. When gentian violet is metabolized under anaerobic conditions, it forms leucogentian violet possibly via the formation of a carbon-centered free radical. Formation of this

carbon-centered free radical has been observed in mammalian systems. This free radical is in turn further reduced to leucogentian violet and subsequently leuco-pentamethyl-pararosaniline, which may also be formed via reduction of penta-methylpararosaniline - a metabolite in the oxidative metabolism demethylation pathway described earlier.

Lastly, in microbial metabolism studies, gentian violet has been shown to be metabolized to Michler's ketone, which is also a carcinogen.

In summary, oxidative metabolism of gentian violet involves the production of the carcinogen formaldehyde with each n-demethylation reaction, and likely also a nitrogen-centered free radical, as well as the fully demethylated carcinogen C.I. Basic Red 9. Reductive metabolism to leucogentian violet is thought to involve the production of a carbon-centered free radical. And a product of microbial metabolism, the carcinogen Michler's ketone, may be produced by intestinal microflora.

Back to Dr. Sun.

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DR. SUN: Gentian violet has tested positive for a number of genotoxicity endpoints including: Mutations in salmonella and E. coli; DNA damage in bacteria and

mouse lymphocytes; chromosomal aberrations and chromosome breakage in various human and mammalian cells; binding to chromosomes in human cells, binding to bacterial, bacteriophage, and isolated calf thymus DNA, and binding to synthetic polynucleotides, and; gene amplification in the SV-40 transformed hamster cell line.

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DR. SUN: Several gentian violet metabolites have also tested positive in genotoxicity assays.

Pentamethyl-pararosaniline chloride is mutagenic in bacteria and bacteriophage, and binds to calf thymus DNA. Leucogentian violet and leuco-pentamethyl-pararosaniline are mutagenic in salmonella. The two tetramethylpararosaniline isomers are mutagenic in salmonella and E. coli.

Formaldehyde C.I. Basic Red 9 as Michler's ketone are all genotoxic carcinogens. As Dr. Ricker mentioned, C.I. Basic Red 9, and Michler's ketone are microbial metabolites of gentian violet, and may be produced by intestinal microflora.

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DR. SUN: We compared the genotoxicity and carcinogenicity of gentian violet to seven structurally related chemicals. Six of these comparison chemicals are -- have a triphenylmethane core, while the 7th,

Michler's ketone, carries a diphenylmethane structure.

Michler's ketone was included because it is a microbial metabolite and can be a precursor of gentian violet synthesis.

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DR. SUN: This table compares the findings from genotoxicity in animal cancer studies for gentian violet, and the seven structurally-related chemicals. You can see that in the three columns under the genotoxicity heading, all seven comparison chemicals were tested for mutagenicity, and all except methyl green were mutagenic.

Three comparison chemicals were tested for effects on chromosomes and were positive, and all seven comparison chemicals were tested for DNA damage or DNA binding, and all except methyl green were positive.

The next column shows that for each of the comparison chemicals that have been adequately tested in animal cancer bioassays, increases in tumors have been observed. The last column identifies the tumor types or sites that were increased. Common tumor sites observed with gentian violet and one or more of the four comparison chemicals with adequate studies include:

Hepatocellular tumors observed with C.I. Basic Red 9, leucomalachite green, and Michler's ketone; thyroid follicular cell tumors observed with C.I. Basic Red 9; and

Harderian gland tumors also observed with C.I. Basic Red 9.

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DR. SUN: We also reviewed the ToxCast high-throughput screening data for gentian violet. It was active in 273 assays out of 794 tested assays. These 273 assays covered 17 different biological processes or intended target families.

We then used IARC's mapping table that maps ToxCast assays to the key characteristics of carcinogens, and found that 72 of the assays that gentian violet is active in, map to five of the 10 key characteristics.

These five key characteristics are shown here in the chart. Each bar indicates the number of assays gentian violet was tested for for that particular key characteristic, and the filled portion of the bar indicates the number of active assays. For example, the bar on the far left shows that gentian violet was tested in nine assays that mapped to the key characteristic 'is genotoxic', and it was active in seven.

The bar on the far right indicates that gentian violet was active in 39 out of 69 assays mapped to the key characteristic 'alters cell proliferation, cell death, or nutrient supply'.

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DR. SUN: We organized the proposed mechanisms of action of gentian violet according to the IARC's key characteristics of carcinogens shown in the left column here. The characteristics highlighted in yellow are the ones that gentian violet has evidence for. They are:

Number one, 'is electrophilic or can be metabolically activated', and number two 'is genotoxic'. These have been discussed earlier. In addition, gentian violet tested positive in several ToxCast assays mapped to genotoxicity.

Number Five, 'induces oxidative stress'. Gentian violet has been shown to generate reactive oxygen species in cell-free systems in the presence of visible light, and in horseradish peroxidase-catalyzed reactions. Findings from several ToxCast assays also support induction of oxidative stress and activation of cellular antioxidant response.

And number eight, 'modulates receptor-mediated effects'. Gentian violet was active in 18 ToxCast assays mapped to this key characteristic, including assays showing activation of the androgen receptor, the estrogen receptor alpha, and the thyroid hormone receptor beta.

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DR. SUN: Here is a recap of the tumor findings in animals for gentian violet. Tumors were observed in

two studies in rats and two studies in mice, including statistically significant increases in:

Hepatocellular tumors in male rats and male and female mice; thyroid follicular tumors in male and female rats; earlier onset of mononuclear cell leukemia in female rats seen at 18-month interim sacrifice; Harderian gland tumors in male and female mice; type A reticulum cell sarcomas, which is now likely histiocytic sarcomas in the bladder, ovaries, uterus, and vagina in female mice; also increases in mesotheliomas of the testis and epididymis in male rats; and clitoral gland tumors in female rats.

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DR. SUN: In addition to the animal tumor findings, we presented the following other relevant data.

During metabolism, carbon- and nitrogen-centered free radicals can be formed. Carcinogenic metabolites include formaldehyde, C.I. Basic Red 9, and Michler's ketone. A number of other genotoxic metabolites can also be formed.

Gentian violet may act via multiple mechanisms. It is a direct-acting electrophile that reacts with DNA and other nucleophiles. Some metabolites are also electrophilic. It is genotoxic. There is evidence suggesting that gentian violet induces oxidative stress. And ToxCast data indicates that gentian violet modulates

receptor-mediated effects.

Finally, gentian violet shares structural similarities with seven chemicals. Six of these comparison chemicals also test positive for genotoxicity. Two chemicals C.I. Basic Red 9 and Michler's ketone are carcinogens on the Proposition 65 list. Three comparison chemicals also induce liver tumors, and one also induces thyroid and Harderian gland tumors.

This concludes our presentation today. Thank you.

COMMITTEE MEMBER EASTMOND: I was going to say welcome back. And Tom is here so he's going to take over.

CHAIRPERSON MACK: Oh, you're going to do it again in a minute.

Thank you, Dr. Sun. Thank you, Dr. Ricker. I was pleased to see that you used Martin's list of potential predictors. I'm not sure that they're all that predictive always, but it's -- it's -- I think it's a good addition.

So, David.

COMMITTEE MEMBER EASTMOND: Well, I think, first, did you want to ask do we have general questions for the -- on the presentation and then we'll turn it over.

CHAIRPERSON MACK: Are there any questions, please? Does anybody have any questions.

BOARD MEMBER EASTMOND: I have a couple.

COMMITTEE MEMBER McDONALD: Yeah, Dr. Ricker, I was wondering if you would talk a little bit about the absorption comparing the rat versus the mouse, at least the NCT -- NCTR studies suggested that the mice had a much greater absorption than the rat, is that your reading of the information?

DR. RICKER: I would have to double check on the paper, but it might be that mice had higher, but it wasn't exceptionally higher. It could have been. Still they are both below 10 percent, I think. I think female mice may have -- may have had higher absorption.

COMMITTEE MEMBER McDONALD: Thank you.

DR. RICKER: But overall, it indicates that absorption is poor and that a large part of the ingested dye remains in the stomach and intestine.

COMMITTEE MEMBER EASTMOND: I have a question. There was quite a bit of toxicity seen in the bioassays, certainly in the rat, maybe the mice. And there was one case in the males, I guess there was some reduced body weight gain. Were there discussions among you about the potential significance of those changes?

DR. RICKER: I'm not sure. Would you mind --

COMMITTEE MEMBER EASTMOND: I mean, I -- well, I can bring it up when I make my comments.

DR. RICKER: Martha can -- yeah.

COMMITTEE MEMBER EASTMOND: I can do it. Just that typically -- I mean, there was really substantial toxicity seen in that 24-month study with the rats, and possibly with the mice. And so when you see that, you start looking at, you know, is how do you evaluate these results? On one hand, not enough animals survived to the end of the test, so you would say that assay may not be as sensitive.

On the other hand, the animals are under considerable physiological stress, because a significant number of them are dying early. And so you know that raises questions about sort of the dosing and acceptability of the dosing. I mean, I've -- I've come to my resolution on that, and I'll comment later. But I didn't know if that had been a discussion that had come up with in your group.

DR. SUN: In the male -- in the male rat study, the mortality was increased after week 95, which is later in the study. In the female rat study, the mortality was seen after year one. And NCTR attributed the mortality to the mononuclear cell leukemia.

COMMITTEE MEMBER EASTMOND: That's the key point.

DR. SANDY: And I'll also add that loss of body weight, I believe that was in -- I remember -- I don't

remember which study that was. But typically, if animals are -- there's a treatment-related decrement in body weight, that is often associated with a lower rate of -- in the controls or of spontaneous -- you know, of tumors. So we can look at that. We tried to discuss it.

COMMITTEE MEMBER EASTMOND: Okay. Thanks.

COMMITTEE MEMBER DAIRKEE: Any thoughts on how the absorption might be different from the food intake in the animals studies as opposed to more of a dermal contact in human and -- a human situation?

DR. RICKER: There were no studies talk -- you know, addressing that. Generally, it's believed that gentian violet may be more easily absorbed compared to similar dyes, just because it's a smaller molecule and appears to be -- have more neutral charges. But we didn't identify any dermal studies.

COMMITTEE MEMBER DAIRKEE: Thank you.

CHAIRPERSON MACK: Go ahead David. Did you --

DR. SANDY: Excuse me, Dr. Mack, there may be another question?

CHAIRPERSON MACK: Wait a minute.

COMMITTEE MEMBER STERN: No. My question was exactly the same. My question was the dermal absorption, if -- there wasn't any mention in the literature, but I was wondering if you had any insights on that, but you

already answered that. But there's no data right to support what gets absorbed?

DR. RICKER: Well, the only -- the only study that might address -- it's not a study. It was a review paper that talked about application of gentian violet as a wound dressing. And generally, it's believed it's not absorbed.

COMMITTEE MEMBER STERN: Not absorbed.

DR. RICKER: Yeah, it seems -- I've forgotten the -- I think -- I think -- yeah, I don't -- it wasn't very -- you know, it was sort of just a comment in a review paper of 2016. But it's generally believed to not be released from the wound dressing, and that may be related to how the wound dressing is constructed.

DR. SANDY: But we don't have data. It's just --

DR. RICKER: Yeah, we don't --

DR. SANDY: There are no studies.

DR. RICKER: Yeah. There's no data to support either way.

COMMITTEE MEMBER EASTMOND: I might mention it is a cation. And it's a fairly large molecule, so you would not generally expect much dermal absorption, because it has a charge on it.

DR. SANDY: If I could, I'll just add though that it is used -- you know, it's for staining bacteria. The

Gram method -- so it does get into cells.

CHAIRPERSON MACK: Joe.

COMMITTEE MEMBER LANDOLPH: I think it was a great presentation. The HID document was very clear to me. Well written.

I just had one question. Was -- is the gentian violet is equal to crystal violet, is that what I heard Dr. Ricker say, is that a correct statement?

DR. RICKER: Yes, it's synonymously used in the literature. And sometimes we find other -- others call it methyl violet. But crystal violet and gentian violet are --

COMMITTEE MEMBER LANDOLPH: Are the same molecule.

DR. RICKER: Yes.

COMMITTEE MEMBER LANDOLPH: Yeah, I request if you could just state that very simply in the executive summary and somewhere in the introduction, because I had to hunt for that. It wasn't stated so clearly in the HID. If you could do that I'd appreciate it.

Thank you.

DR. RICKER: We'll do that. Thank you.

CHAIRPERSON MACK: All right. David.

COMMITTEE MEMBER EASTMOND: All right. Thank you. I would also like to express my appreciation to the

OEHHA staff for the -- summarizing things so nicely in the document, and in the presentation.

I -- this appears to be a pretty straightforward compound in many respects. As I looked at this, there are clear dose-related increases in thyroid follicular cell adenocarcinomas. They were seen in both male and female rats in the 24-month study. These increases were significant by a trend test as well as pairwise comparisons. And there was significant increase seen in sort of combined basically thyroid follicular cell adenomas and adenocarcinomas seen in both the males and female rats. So that's one where I think there's a strong response seen in both males and females.

There's also a significant dose-related increase in hepatocellular adenomas seen in the male rats. And modest increases were all seen at the two highest doses in the females. So there appear to be substantial evidence for carcinogenicity in my mind. And those were the two tumor types in the rat I put the most emphasis on.

I saw that there had been pretty high mortality. And that starts raising concerns, because as I indicated, you get trade-offs. If there's too many animals die early on the study, the study is not very powerful because they don't last long -- the animals don't live long enough to see the tumors.

On the other hand, animals that die early in that treatment-related fashion tend to be under a tremendous sort of physiological stress. And so then you would argue well this may have exceeded what would be considered sort of a maximum tolerated dose.

The key point on this, and I spent some time in chasing it down, is that most of the animals that died earlier died because of mononuclear cell leukemias. And so the other deaths were apparently, as described, spread across the other treatment concentrations and tissues. So there wasn't any obvious pattern there. So that kind of alleviated my concern on that particular concern about maximum tolerated dose and toxicity, at least in that.

In the mice, again, you've got clear dose-related increases in tumors seen in male mice, hepatocellular adenomas and carcinomas in female mice, adenomas carcinomas, and then Harderian tumors plus these histiocytic sarcomas or reticulum cell sarcomas in four separate issues.

So again, there's strong evidence in the mice. Again, the same issue came up with toxicity, and a lot of the toxicity was apparently due to liver cancers or responsible it said for 50 percent of the tumors of the high -- 50 percent of the deaths at the high dose were due to liver tumors. So that alleviated some of my concern

about the doses there, at least as toxicity occurring.

Coming on to the genotoxicity was kind of intriguing for me. Now, for those of you that didn't look at this, a lot of these studies were done many years ago, so they're quite old. And so I went to a few of them that seemed to be newer studies that I had sort of more confidence in, and looked at them, or chased down a couple of the old ones that I thought were important.

So gentian violet is, what I would consider, weakly mutagenic in the Ames test. It is significant, but it's not a strong positive. Increases tend to be between sort of 2- and 2.5-fold, but there's a dose-related trend and it's high enough that you'd call it positive.

The -- it was clearly clastogenic, so it caused chromosomal breakage in vitro in mammalian cells, at higher concentrations. The intriguing thing -- in fact, this was -- it must have been William Hou's dissertation I would bet. He did about 10 different cell lines -- was that when they added S9, which is used as a -- for metabolic activation, the clastogenicity went away. And they didn't need the co-factors either.

So it suggests to me that it's actually binding to the protein, which suggests -- so that's in vitro where you're seeing these sort of positive things. In vivo, they didn't see any evidence in certain bone marrow tests

for chromosomal damage. In vivo, there were a couple of studies done. And that may be as follow-up study for the in vitro cited genetic studies, but it doesn't really address the mutations that we're seeing.

So, I mean, I think there's certainly evidence that it's genotoxic -- a genotoxic compound, which is consistent with sort of the onset of tumors, and one of the mechanisms which is associated with carcinogenesis.

And then you can see -- with similar type structurally-related compounds, you can see generally somewhat similar genotoxic and carcinogenic profiles.

So as sort of bottom line on this is that I think this is clearly carcinogenic, and something that, I guess, we'll talk -- would -- that would be listed under Proposition 65.

Tom, do you want to follow up?

COMMITTEE MEMBER McDONALD: Yes, as the second discussant. I would also like to thank OEHHA staff.

Get closer. Is that better?

Great.

I would also like to thank OEHHA staff. I thought the compilation of the carcinogenic evidence was very good about gentian violet, which I may refer to as GV.

(Laughter.)

COMMITTEE MEMBER McDONALD: I particularly liked the comparison of the structurally-related compounds and metabolites. I thought that was well done. And I also really found it helpful the discussion around the tumor biology providing context, putting the historical control data right there, so it was easy for review.

Just one thing for the future that I think would be helpful. I know that you had cited JECFA's review as part of your genetox section, but if you could provide at least the citations as part of the full compilation of the original papers, if you're not going to cite them yourselves, it would just helpful. I had to go look them up and just facilitate review.

You know, gentian violet is clearly genotoxic in vitro. One issue that I went back and forth in my mind was -- that wasn't discussed in great detail was this issue of cytotoxicity. Gentian violet is cytotoxic, and very much so in some cell systems. You see this clinically with ulcerations in children's mouths, the hemorrhaging and necrosis in the liver of the treated mice.

And in vitro systems, especially in the in vitro -- the genetox studies you see that, I think, as you described, Dr. Eastmond, where you have this pull and push between viability and mutagenicity.

It's also -- this compound is really a potent mitochondrial toxicant. It's -- there's some recent papers, which shows that it inhibits mitochondria, which is going to lead to apoptosis, cell death, and then of course the compensatory inflammation oxidative stress and so forth.

And there's been a lot of recent publications that in human fibroblasts and in breast cancer cells that you have reduced viability down in the nanomolar range. So it's really quite a potent cytotoxin.

Just out of interest, there's been sort of a resurgence of this compound as a therapy. I saw that because of these mitochondrial toxicity features, that clinicians are now looking at it as a -- as an antineoplastic agent, treating a number of things. But that -- so anyway, it really doesn't feed into the hazard ID, so much. It's more mechanism and dose response, but it was really interesting to try to tease out what's going on with respect to DNA damage versus cytotoxicity and compensatory proliferation.

So I think you nicely stated the problematic human data, and the early animal cancer studies. There were actually two, one in the 1930s and one in the 1940s. Very limited reporting there. But I think it's interesting to note that at least the original author call

was not inconsistent with the later studies. So I think that, you know, at least should be stated.

The later animal cancer studies, the lifetime studies in the rats and the 24-month in the mice. One thing I want to say about the rat study, you know, when you -- when you treat starting 80 days prior to mating all the way through gestation, lactation, dose, the pups as well, all the way through life, you get a much greater spike of dose in early life, almost two- to three-fold higher dose in those early life. And with a cytotoxic compound you kind of wonder well, does that -- does that really play into what you're seeing. But again, like I think Dr. Sandy noted, that the body weight again and food consumption in these studies were not appreciably different from controls. So I'm not really worried about enough of the dose getting in systemically to create -- create an issue, so that there was less than 10 percent there.

Significant increase in thyroid tumors. And I wanted to say one thing about the mononuclear cell leukemias. They were not statistically -- they weren't statistically significant at end of study, but they were at 18 months. And NCTR had done a really nice statistical analysis where they had shown that there was a strong statistical association with onset of leukemia and dose,

as well as mortality by leukemia and dose. So I think that was an important add to make.

So the -- there was a much greater response in the mouse. And I think that may be due to the greater absorption of gentian violet. NCTR suggested about a three- to four-fold greater uptake, so that may be part of it, or it may just be susceptibility. Again, there was very little progression seen of the lesions, nothing seen at 12 months, some at 18 months, but all end-of-life observations.

In the mice, there was -- if you looked at the clinical chemistry data, all of the liver enzymes were significantly up, suggesting stress to the liver, again, is this cytotoxicity, is it DNA damage, is it both? But clearly, you've got these mechanisms going on in the liver as indicated by the clinical chemistry data.

I think -- I did want to make some points. I think we covered -- on genetox, I did want to make some points. This clearly binds to DNA. It's clearly clastogenic. With respect to the Ames test, yeah, I felt the same way as Dr. Eastmond when I look at this. You can see the cytotoxicity where it's barely a doubling before you get loss of viability, and so...

And then looking at the -- there's lots of evidence of DNA damage in vitro, but minimal in vivo.

There were actually three studies and none of them showed a response. I just want to make one point about those studies. There was a chick embryo study, high toxicity, no sister chromatid exchanges. There was a four-week drinking water study up to 8 mg per kg of gentian violet in the drinking water with no chromosomal damage. And then there was mouse lymphocytes looking at DNA damage. But that was a tail vein injection up to 6 mg per kg.

Now JECFA had suggested that the doses are much lower than what was done in the cancer studies, so we can discount those in vivo studies. But, you know, if there really is a low absorption rate, maybe this tail vein injection being an IV directly into the systemic blood. Maybe that's more relevant to the cancer.

So I just wanted to point that out as something of interest. I really would have liked to have seen somebody do a proper in vivo genetox study at the doses that were used in the bioassays, the cancer bioassays.

As I stated before, I really liked the comparison to structurally similar molecules. I think there's a strong weight of evidence there. And I'm curious to hear what my other Panel members feel about the ToxCast data. You know, you all probably look at this type of data more frequently than I do. But there clearly seem to be a lot of DNA damage and cytotoxicity, oxidative stress, the same

sort of competing mechanisms.

But anyway, that's my comments. And I would agree that I think it's a proposed listing.

CHAIRPERSON MACK: Thanks, Tom.

Now, let's go through the -- I was going to start with Jason. Do you have any comments?

COMMITTEE MEMBER BUSH: I don't have anything to add.

CHAIRPERSON MACK: Okay. Shanaz.

COMMITTEE MEMBER DAIRKEE: I just wanted to thank the staff for providing us with the ToxCast data. It is very complex, high-dimensional data, and difficult to understand. But it came through very clearly from that -- certain things came through very clearly, the genotoxicity, went very well with the P53 going up in several assays. But I -- there's a caveat here, that listing so many assays as being active, and not having clarity even in the ToxCast data, whether the activity was in the positive direction or the negative direction.

And by that I mean that when P53 goes up, cell proliferation goes down. So even if you have an active assay, it doesn't mean that the cells are proliferating. They are not proliferating as also an active assay in the ToxCast system.

So I think my colleague here made a very good

point that the cytotoxicity is -- comes across much more in the ToxCast assays than the carcinogenicity aspect, so -- but overall, it is very clear that it's a genotoxic compound. And that's all I have to say.

CHAIRPERSON MACK: Thank you. Michelle.

COMMITTEE MEMBER LA MERRILL: Thank you. I thought the material was very clear and really helped facilitate my review. I don't really have much additional to say. But I do think that it's strong to note that there is multiple tumor sites in both sexes of two mammalian species. And that even putting the ToxCast aside, it looks like, you know, key characteristics are represented in there by about four different key characteristics. And I did find it helpful that although the in vivo data was a bit sparse, that we did see presence of the oxidative metabolites, in that helpful table where you indicated which carcinogens were -- or, excuse me, which of those metabolites formed tumors that were -- that related to the parent compounds.

Thanks.

CHAIRPERSON MACK: Joe, do you have other comments?

COMMITTEE MEMBER LANDOLPH: Yeah. This was a relatively easy one for me. In fact, after awhile, I got tired of reading all the positives.

(Laughter.)

COMMITTEE MEMBER LANDOLPH: So in my, you know, role as a senior member who -- help teach a little bit here. There is an overwhelming amount of data here. There's no doubt in my mind whatsoever that this is a metabolizable DNA-binding genotoxic metabolite. It's positive in many different systems for in vitro genotoxicity.

I was looking at the -- the number of tumor sites is one, two, three, four that are very strong, and another five, six, seven, eight, nine, ten organs that it causes tumors in in male and female mice and rats. So this one doesn't really require much thought. I mean, we've had chemicals that were kind of marginal. And this has like about 20 times as much evidence. So I don't have any problems with this at all.

The ToxCast data, I think is kind of peripheral. I hate to be denigrating about it, but I think it's kind of marginal. I like the solid endpoints, like the genotoxicity, the DNA binding, the mutagenesis, the clastogenesis, the tumorigenicity data. I think it's clear EPA want to use this ToxCast data, but I'm not really wild about it. I think if you're going to put something regulated into the legal arena, you better have solid data. And that ToxCast data really doesn't impress

me that much. It never did as I've seen it develop.

So my vote for this would be overwhelmingly that it is shown by the standard methods, scientific methods, to be carcinogenic.

CHAIRPERSON MACK: All right.

COMMITTEE MEMBER STERN: Yeah. I don't have a lot to add. I agree the documents were incredibly clear, so thank you for that. I learned a lot. It was wonderful to read. I think what I found very compelling was that the chemotypes show the localization of the tumors match the key localization for gentian violet. So I think that that's a very compelling argument on top of everything else.

And, yeah -- and sorry, I lost my train of -- I was going to say something else that I found important, but now the thought escaped me.

But I agree that it has to be -- the recommendation has to be to list it, because I think it's a compelling argument that it's carcinogenic.

Oh, sorry, I remember my thought. The other thing that I thought was compelling that hasn't been mentioned yet is that many, at least three of the key metabolites of gentian violet are known to be potent carcinogens, like formaldehyde, for example. So I think that makes a very compelling argument that overall it's

carcinogenic.

CHAIRPERSON MACK: Thank you. Peggy.

COMMITTEE MEMBER REYNOLDS: I also want to thank the staff for always a very nice and comprehensive review, and by the way, for being so diligent to try to find human health evidence.

(Laughter.)

COMMITTEE MEMBER REYNOLDS: And I think that these two reports, they weren't really studies, that are over 30 years old were interesting, not particularly informative. I think the -- the Brazilian study, the investigators very clearly said they were really trying to see whether people could self-report exposure, as opposed to really doing an outcome study.

I think it's interesting that these reports are over 30 years old, and we haven't heard anymore about this. But nonetheless, in the absence of particularly compelling human health evidence, I think the other evidence that was presented is very compelling. And I thank you.

CHAIRPERSON MACK: Luoping.

COMMITTEE MEMBER ZHANG: Okay. As most of the Panel already say, you know, the -- I really think today this presentation I would say is one of the best --

(Laughter.)

COMMITTEE MEMBER ZHANG: -- while I'm being here. Very clear, particularly the metabolism. You know, it's complicated structurally. But you presented the way it's very easy for everybody to follow. Particularly also, I think, you know, you mentioned that like formaldehyde. So everything if it's already identified as a carcinogenic compound, it's presented very clearly. So I really like that. So even though everybody was saying, I still want to have my chance to -- to acknowledge.

And another thing I want to also say is you included the key characteristics, and trying to, you know, put that into. I think that's -- it's a good way. And also, I really like that. I hope you can continue to apply that idea into our process when we're trying to identify the carcinogen.

Back to one point is ToxCast assays. So I actually think, you know, always -- you heard, you know, some members, but I think it's a good idea to just see what other assay has been tried. I was actually surprised that, you know, they even tested for this gentian, you know, violet. You know, I don't now how they pick it up.

But I think if they already test it, and there's some data, and then you bring that to here, and the first to compare what you already found, I still think this approach still good.

I mean, we're not really trying to only using the ToxCast data to make our judgment, but it's good to bring that somebody else already looked at this, and this is what we found. And then in comparison with our KC, you know, key characteristic data, I still think it's a very good approach. So I gave you a really, you know, plus, plus, plus for that.

(Laughter.)

COMMITTEE MEMBER ZHANG: So back to -- everybody already saying this is very clear carcinogen. So there's no doubt. But the only thing one -- you know, following my fellow member Peggy, there's only one human study, okay, for this, right? It's a hospital based. It's another very -- I know we're not focusing on that, but I'm still thinking -- I was just wondering when you presented the human data, I was trying to find it, you know, back to the original study, but I couldn't.

So one thing I thought if 26 of the 37 reported, you know, had a single exposure, had some kind of benign or malignant lesions or cancers, so I actually -- really, my mind was thinking about -- how about another site. The rest if they don't have or how many they have. So I would -- but I was really trying to find out, but you know, I don't know if originally they didn't -- yeah, so I did a quick calculation. That's like 70 percent of, you

know, 26 of the 37. That's really high.

So but anyway, I'm just wondering about that only human data. I know you won't create that one, but anyway.

COMMITTEE MEMBER REYNOLDS: I just -- I just want to -- I just want to add that it was really nice to get some translations from the Portuguese to be able to actually read those original comments. And I didn't mention the German case study, but that was intriguing, but a case study.

COMMITTEE MEMBER ZHANG: Yeah. Yeah. Anyway. Okay. So even I find --

CHAIRPERSON MACK: Anybody else have any afterthoughts?

David.

COMMITTEE MEMBER EASTMOND: I have one additional question for the OEHHA staff. I talked about DNA binding. Do you know if that was covalent binding DNA or was that sort of binding like intercalation where you get staining? Because that's the one thing that I wondered about.

COMMITTEE MEMBER McDONALD: I figured that out as well.

CHAIRPERSON MACK: Any others?

DR. SUN: I think the early studies showed that it binds to the AT sites in the DNA. And I don't believe they found covalent adducts.

COMMITTEE MEMBER EASTMOND: Okay.

CHAIRPERSON MACK: We haven't had any volunteers from the public to make comments. Gary, are you motivated?

(Laughter.)

CHAIRPERSON MACK: Does anybody else want to step up and make remarks?

If not, then we'll go to the voting procedure.

So the words that I am supposed to be very careful about reading are, has gentian violet been clearly shown through scientifically valid testing, according to generally accepted principles to cause cancer? All those voting yes, please raise your hand.

(Hands raised.)

CHAIRPERSON MACK: All of those voting no?

(No hands raised.)

CHAIRPERSON MACK: So the decision is unanimous. We have decided that it does in fact cause cancer, and it requires listing. Now, do you want to take a break for a little while?

DIRECTOR ZEISE: Five minutes.

CHAIRPERSON MACK: Okay. You can use that. Okay fine.

(Off record: 11:15 a.m.)

(Thereupon a recess was taken.)

(On record: 11:32 a.m.)

CHAIRPERSON MACK: I guess we can get started again. Are you prepared?

Okay. Go ahead. Oh, wait a minute. No. Lauren has some --

DIRECTOR ZEISE: Yes. I have some corrections to the introductions of the Panel. First, I gave Joe an early retirement, so Dr. Landolph has not retired.

(Laughter.)

DIRECTOR ZEISE: And so that's the first thing. And the second thing is that Dr. Reynolds is now with the Department of Epidemiology and Biostatistics at the University of California, San Francisco.

So thank you.

COMMITTEE MEMBER DAIRKEE: One more.

DIRECTOR ZEISE: And one more.

COMMITTEE MEMBER DAIRKEE: My last name is Dairkee. Dr. Dairkee.

DIRECTOR ZEISE: Dairkee. And Dr. Dairkee. Thank you.

CHAIRPERSON MACK: All right.

DR. SANDY: Okay. Thank you, Dr. Mack. This is Martha Sandy.

So the next chemical that you're going to hear about is one that's hard to say,

n-nitrosohexamethyleneimine. We brought that to your Committee during -- in 2009 for prioritization. So it's -- it was awhile ago. I wanted to also point out because this chemical has a lot -- a number of bioassays, we used a format with mostly tabulation of those bioassays in the -- the table format was a little different, and we're happy to hear if you want to give us some feedback on that in your comments.

You'll be hearing from three different staff, Dr. Feng Tsai, Dr. Jennifer Hsieh, and Dr. Gwen Osborne.

(Thereupon an overhead presentation was presented as follows.)

DR. TSAI: Good morning. My name is Feng Tsai. And today we are here to present the evidence on the carcinogenicity of n-nitrosohexamethyleneimine. This presentation is an abbreviated version of the data that were reviewed in the hazard identification document provided --

DIRECTOR ZEISE: Excuse me, Dr. Tsai, could you speak just a little bit more into the microphone and a little louder?

--o0o--

DR. TSAI: Sure. So throughout our presentation, we'll use the shortened -- shorthand term NHEX to refer to this chemical. NHEX is a heterocyclic nitrosamine that is

formed by the reaction by a secondary amine and a nitrosating agent. NHEX is not known to occur naturally.

NHEX has been reported to be a contaminant in a prescription drug for diabetes called Tolazamide. NHEX may also form in the acidic environment of the stomach in patients taking this drug with nitrite from diet.

There is little information on current use of NHEX. Historically, it has been used in industrial chemical synthesis. It is also used as an explosive in ejector seats of military jets.

This chemical has not been reviewed by any Proposition 65 authoritative bodies. The European Chemical Agency, ECHA, has classified this chemical as a category 1B carcinogen, meaning NHEX is presumed to have carcinogenic potential for humans, largely based on animal evidence.

Like other nitrosamines, NHEX has a nitroso group circled in red in the chemical structure shown here. The alph-, beta-, and gamma-carbon positions are also labeled.

--o0o--

DR. TSAI: No human data were identified in the literature search for NHEX. There's a rich set of animal studies with 33 cancer bioassays identified. This table summarizes a number of exposure routes, strains, and experiments by species for the bioassays. NHEX was

studied in three species, mice, rats, and hamsters in both sexes, and often using multiple exposure routes and strains.

Information on the study design and study finding of each of the 33 bioassays is presented in the hazard identification document. In the interest of time, today we'll only summarize key findings from these studies by species, and present detailed information from two or three studies for each species as examples.

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DR. TSAI: This slide shows the overview of the bioassays in mice. A total of 15 studies were conducted in eight strains with different exposure routes, including drinking water, gavage, and subcutaneous injection.

Additional study design information, not shown on this slide, including the following: Small numbers of treated animals were used in these bioassays, ranging from 10 to 20 animals per treatment group. All 15 bioassays included concurrent controls.

A high level summary of the treatment-related tumor findings from these studies is presented here. Tumor types shown in the red color indicate rare tumors in untreated mice, and asterisk represent statistically significant increase in tumor incidence at P equal to 0.05, either by pairwise comparison with control or by

trend test.

In NHEX-treated mice, statistically significant increases were observed in both sexes of tumor of the oropharynx, esophagus, lung, three types of liver tumor, forestomach, glandular stomach, and reticuloendothelial lymphoma. Several of these significantly increased tumors are also rare. In addition, increases in rare nasal cavity tumor were observed in treated female without reaching statistical significance.

Next, I'll present two examples of mouse bioassay.

--o0o--

DR. TSAI: This is the first example. Male NZO mice were treated with NHEX via drinking water, five days a week for eight weeks, and observed until death or killed when moribund. The first two columns show the tumor site and tumor type. R in the tumor site or tumor type column indicates the tumor is rare in untreated animals.

An unusual tumor grouping of oropharynx was used by these authors, and included tumors of the nasal cavity, tongue, and larynx, as well as the oral cavity and pharynx.

Increases in malignant tumors or combined benign and malignant tumors of multiple rare tumors shown in this table were statistically significant by pairwise

comparison with controls. These rare tumors are the oropharynx, esophagus, liver cholangioma and cholangiocarcinoma, forestomach, and glandular stomach. Statistically significant increases of other malignant tumors were also observed, specifically hepatocellular carcinoma and reticuloendothelial lymphomas.

--o0o-- sighs

DR. TSAI: This shows another example of a mouse bioassay. Female SENCAR mice were gavaged with NHEX in corn oil twice a week for 30 weeks. Control animals received vehicle only. Animals were observed until death or killed when moribund. In cases where control incidences of tumor types were not reported, shown as NR here, we used the incidence for all tumors at that specific site to perform the pairwise comparison.

For example, the number of lung adenoma in controls was not reported. We used the total number of lung tumors at 1 out of 20 to conduct a pairwise comparison for lung adenoma. The same approach applied to liver or forestomach tumors.

Statistically significant increases in malignant, or benign, or a combination of benign or malignant tumors were observed in the lung, liver, and forestomach. Note that the total liver tumors, 12 out of 20, were reported in the paper by Strickland et al. We usually do not sum

up tumors from different cell types. Forestomach carcinomas are rare in mice. Increases in benign and malignant nasal cavity tumors and benign esophageal tumors, all of which are rare, were also observed.

I've only presented two examples of the 15 bio -- mice bioassays. Detailed information on all 15 studies can be found in table 4 of the HID.

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DR. TSAI: This is an overview of the rat's bioassay. NHEX was administered in three strains of rats through drinking water in six studies. One additional subcutaneous injection study that was reported in a short German abstract with limited information was not included in this slide.

Small numbers of animals, 15 to 20 per treatment group, were used in these rats bioassays.

Among the six bioassays, one study included a concurrent control, one study used colony control, and four bioassays did not include control. However, high incidences of rare tumors were observed repeatedly in these experiments without control.

For example, in two drinking water studies conducted by Goodall et al., 100 percent of the treated males and 73 percent of the treated females developed rare hepatocellular carcinomas or rare liver hemangiosarcomas.

A high-level summary of the tumor finding in these rat studies is shown here. In NHEX treated rats, statistically significant increases in rare tumors included: tumors of the rare nasal cavity in males; and tumors of the esophagus, hepatocellular adenoma and carcinoma, and liver hemangioma and hemangiosarcoma in both sexes.

In addition, increases in rare nasal cavity and tongue tumors were observed in females, without reaching statistical significance.

Two rat studies will be shown next as examples. Details can be found in table 5 of the HID.

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DR. TSAI: This is the first example. Male SD rats received NHEX via drinking water, five days a week for 30 weeks, with a total dose of 330 milligrams per animal. This study did not include a concurrent control group. The author refers to the spontaneous tumor incidences from a continuous series of unexposed male rats from the same animal colony maintained in the same facility as colony control.

Statistically significant increases in malignant tumors were observed in the nasal turbinate and for two different cell types in the liver. All are rare tumors.

Rare esophageal papillomas and carcinomas were

also seen, and the increase in papillomas was statistically significant.

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DR. TSAI: This is another example of a rat bioassay. Female male F344 rats received NHEX in drinking water five days a week for 28 weeks, and observed until death or killed when moribund.

Statistically significant increases in rare malignant tumors were observed in the esophagus and in two different cell types in the liver. The combined incidence benign and malignant esophageal tumors was also statistically significant.

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DR. TSAI: 11 NHEX bioassays were conducted in Syrian golden hamsters including seven experiments by subcutaneous injection and four experiments by transplacental exposure as a result of subcutaneous injection of the pregnant dams. These transplacental studies were designed to investigate whether the prenatal life stage is more susceptible to NHEX than the parent generation. And the doses used in these studies were characterized by the author as low or non-carcinogenic. Three transplacental studies used a single dose of 10 milligrams per kilogram. The doses used in the four studies were two to eight times higher.

A high level summary of the tumor findings in these hamsters is shown here. Because of the special two-generation study involved in the hamsters, we separated tumor findings by exposure routes.

In NHEX-treated hamsters by subcutaneous injection statistically significant increases in rare tumors were observed in the nasal cavity and trachea in both sexes, and in the lungs in males. Rare laryngeal tumors were also observed.

In hamster receiving NHEX via transplacental exposure, no treatment-related tumor findings were found in the single-injection studies. In the multiple-injection study, increases in rare laryngeal and tracheal tumors were statistically significant in the offspring. Similar tumor findings were also observed in the parent generation, reported above in the subcutaneous injection results.

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DR. TSAI: This is the first example from the hamster bioassay. This subcutaneous injection study in males is one of the few available NHEX bioassays that have multiple treatment groups receiving doses ranging from four to 64 milligrams per kilogram. Animals received weekly subcutaneous injections for life. Dose-dependent decreases in survival were observed in three highest dose

groups compared with control. The median survival for the highest dose groups of 64 milligrams per kilogram were only about 18 weeks.

It is possible that animal in this highest dose group may not have lived long enough for tumors to have developed at some sites. Statistically significant increases in combined benign and malignant tumors were observed in the nasal cavity and in the lung in one or more dose groups. Statistically significant increases in benign tumors of the trachea were also observed in all dose groups with a significant dose-related trend.

Increases in laryngeal tumors were also observed. All of these sites are rare in hamsters.

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DR. TSAI: This slide shows the results of two studies in pregnant hamsters. One is a single subcutaneous injection study, and the other administered multiple injections of what was described by the investigator as non-carcinogenic dose of NHEX. The dose of NHEX in the single injection study was 10 milligrams per kilogram. It was administered on different days to different pregnant hamsters in different -- in the treatment groups, and occurred between gestation days 8 to 15.

In the multiple injection study, pregnant

hamsters received anywhere from two to eight injections within the period of gestation days 8 to 15. The total dose of NHEX received by individual animals in the multiple injection study ranged from 20 to 80 milligrams per kilogram body weight.

No tumors were observed in treated females in the single-injection study.

In multiple injection studies, statistically significant increases in rare benign tumors of the larynx and the trachea were observed. In addition, two rare malignant nasal cavity tumors were observed.

Next, Dr. Hsieh will present a summary of the other relevant data.

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DR. HSIEH: Thank you, Dr. Tsai.

I will start with a summary of the pharmacokinetics and metabolism of NHEX. NHEX is absorbed and distributed rapidly, metabolized completely, and excreted in the urine, and in the breath as carbon dioxide.

NHEX can be biotransformed by cytochrome P450 enzymes to form a number of metabolic products:

Although the hazard identification document indicated that 17 metabolites have been detected and identified in mammalian systems, the correct number should

be 18. The additional metabolite is hexamethyleneimine. It was detected in the urine in NHEX treated rats by gas liquid chromatography analysis in the paper published by Grandjean 1976. Seven additional metabolites of NHEX has -- have been proposed, and a number of other metabolites have been detected but not yet identified.

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DR. HSIEH: Now, I will walk you through the metabolism of NHEX, which occurs through a number of different pathways.

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DR. HSIEH: Here is the structure of NHEX. NHEX is metabolized by cytochrome P450 enzyme under a number of pathways, including multiple hydroxylation and denitrosation pathways. As I walk through the different metabolic pathways, chemical names shown in bold indicate metabolites that have been detected in mammalian system. Reactive intermediates are shown in brackets. Question marks indicate proposed reaction.

Let me start initially with three hydroxylation pathways. Several studies show that NHEX can be hydroxylated at alpha-, beta-, gamma-carbon to form alpha-, beta- or gamma-hydroxy NHEX. Beta-hydroxy NHEX and gamma-hydroxy NHEX can be further metabolized to form oxidative derivative. Alpha-hydroxylation appeared to be

the predominant hydroxyl -- hydroxylation pathway. It is also the most well studied pathway. And I'll show you the step involved in further metabolism of alpha-hydroxy NHEX in a minute. Carbon dioxide can be produced in each of these hydroxylation pathways.

Two denitrosation pathways have been proposed. In the first, an electrophilic nitrosonium ion is formed, along with hexamethyleneimine, which I mentioned earlier has been detected in the urine of rats exposed to NHEX. In the second pathway, an NHEX radical, NHEX imminium ion, hexamethyleneimine, which is the ring structure with a double bond in the center of the figure here, and a nitrosonium ion are proposed.

NHEX has also been shown to form epsilon-aminocaprohydroxamic acid.

Now, let's look at the later steps in the alpha-hydroxylation pathway. This pathway is thought to be the primary pathway of NHEX metabolism, and to involve the formation of a several reactive metabolites. These include the formation of NHEX radical and NHEX imminium ion, both of which has been proposed to form alpha-hydroxyl NHEX.

After alpha-hydroxylation, the ring structure is cleaved between an alpha carbon and a nitrogen atom to form diazohydroxide. Diazohydroxide can be further

converted an unstable intermediate carbonium ion metabolite, then by a hydration reaction, recruiting a water molecule to form 6-hydroxyhexanal. After a reduction reaction, 6-hydroxyhexanal is converted to 1,6-hexanediol, and eventually it's metabolized to form carbon dioxide. 1,6 hexanediol can also react with DNA and RNA, as 1,6-hexanediol adducts has been observed in rats exposed to NHEX.

Adipic acid and epsilon-caprolactam can also be produced from alpha-hydroxy NHEX. Epsilon-caprolactam is then metabolized further to carbon dioxide.

In order to recap a number of different pathways of NHEX metabolism, which I have just shown you, here is the whole picture of the NHEX metabolic scheme.

During these biotransformation processes, several reactive electrophilic metabolites have been proposed, including a NHEX radical, a NHEX imminium ion, a carbonium ion metabolite, and nitrosonium ions -- nitrosonium ion.

In addition, formation of the genotoxic and electrophilic metabolite, 1,6-hexanediol, has been demonstrated.

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DR. HSIEH: Now, moving on to genotoxicity studies of NHEX. Available genotoxicity studies in bacteria, in mammalian cells, and in in vivo studies in

Drosophila and rats are summarized here from top to bottom.

In bacteria, NHEX induced base-pair substitution mutations in four salmonella studies, and in one E. coli study in the presence of S-9. In another salmonella mutation assay when the strain was not specified, NHEX was positive in the presence of S-9 and weakly positive in the absence of S-9.

In vitro, NHEX was mutagenic to Chinese hamster lung V79 cells co-cultured with primary rat hepatocytes in 6-thioguanine and Ouabain resistance mutation assays.

In vivo, NHEX was mutagenic in the Drosophila x-linkage recessive-lethal mutation assay. And, following, in vivo exposure to rats, NHEX was found to -- was found to alkylate rat liver RNA and/or DNA in three studies. In another rat NHEX was not -- in another rat study, NHEX was not found to induce DNA single strand breaks or alkali-labile sites, as measured by alkaline elution, in the liver, lung, kidney, or duodenum.

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DR. HSIEH: Only a few NHEX metabolites has been tested for genotoxicity. Beta-hydroxy and gamma-hydroxy NHEX were mutagenic in salmonella mutation assay causing base-pair substitution mutations. As mentioned earlier, 1,6-hexanediol has been detected as covalently bound to

rat liver DNA and RNA in vivo.

Genotoxicity findings of epsilon-caprolactam were primarily negative. Adipic acid was negative in mutagenicity assay in bacteria and in mammalian cell.

No genotoxic studies were found for other metabolites.

Next, Dr. Osborne will present the findings from structure activity comparisons.

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DR. OSBORNE: Okay. So the structure of NHEX is shown in the center. NHEX shares structural similarities with other cyclic nitrosamines. The five chosen for comparison are shown here, several of which are very similar in structure to NHEX but with different numbers of carbons.

Four of the five comparison chemicals are listed as carcinogens under Proposition 65. These are 2,6-dimethylnitrosomorpholine, or DMNM, nitrosomorpholine, or NM, n-nitrosopiperidine or NP, and n-nitrosopyrrolidine, or NPYR.

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DR. OSBORNE: All five comparison chemicals induce tumors in animal cancer bioassays, and, as shown here, each of these five chemicals share common target tumor sites with NHEX in one or more species.

The different tumor sites observed in studies of NHEX are indicated in the column headings across the top of the table, and the different chemicals are presented in each row with NHEX in the first row.

The species that the tumors occur in are indicated in the table with R for rats, M for mice, and H for hamsters. Nasal cavity, larynx and/or trachea, and lung tumors were seen with NHEX in all five comparison chemicals. Esophagus and forestomach tumors were seen with each -- seen with four of the comparison chemicals. With regard to the liver, this slide presents a simplified version of the information in table 13 of the HID, because NHEX induces three different types of liver tumors.

Hepatocellular tumors and vascular tumors were seen in the same species as with NHEX with three comparison chemicals. Bile duct tumors were seen in three different comparison chemicals, but in different species than NHEX.

Not shown here, but discussed in the HID, additional NHEX target sites, namely tongue and pharynx, were each observed with two comparison chemicals and glandular stomach tumors were observed with one.

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DR. OSBORNE: This table compares the findings from genotoxicity studies for NHEX and the five

structurally-related chemicals. All comparison chemicals that were tested for various genotoxicity endpoints were positive. Specifically, all tested comparison compounds were positive for mutagenicity in salmonella and E. coli, and for mutagenicity and/or DNA or chromosomal endpoints in mammalian cels in vitro.

All chemicals that were tested in Drosophila were positive for x-linked recessive-lethal mutations and all that were tested for DNA and/or RNA binding in vivo in rats or hamsters were positive.

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DR. OSBORNE: Quantitative structure activity relationships, or QSAR -- excuse me -- predictions for NHEX have been published by the European Chemicals Agency, known as ECHA. QSAR models predict the toxicity of chemicals by correlating their physical and chemical properties of related compounds to the biological activity quantitatively.

ECHA analyzed NHEX using the QSAR toolbox and several different models in the VEGA QSAR platform. As shown on this slide, the QSAR toolbox and the various carcinogenicity and mutagenicity models within VEGA each predicted that NHEX is a carcinogen and a mutagen.

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DR. OSBORNE: OEHHA has organized the proposed

mechanisms of action of NHEX according to IARC's key characteristics of carcinogens. The ones highlighted in yellow are the characteristics that NHEX has evidence for.

These are, number one, 'is electrophilic or can be metabolically activated'. The evidence comes from several proposed reactive intermediates and an identified metabolite 1,6-hexanediol with evidence of DNA and RNA alkylation in rats.

Also, key characteristic number two, it's genotoxic. The genotoxicity findings for NHEX have already been summarized and are shown here on the slide. In addition, positive mutagenicity findings have been reported for the beta- and gamma-hydroxy NHEX metabolites.

Now, we'll return to Dr. Hsieh for the summary of evidence.

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DR. HSIEH: Thank you, Dr. Osborne.

Now, I would like to summarize the evidence on the carcinogenicity of NHEX.

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DR. HSIEH: Okay. This table summarized the finding from many studies conducted in mice, rats and hamsters. Common NHEX target tumor sites were observed in multiple species, strains, and often in both sexes. In this slide, the target tumor sites are listed in the left

column and the top header row indicated a different animal species. The yellow highlight indicates rare tumor sites.

Now, I will present a summary of tumor evidence from top to the bottom.

First, increases in rare nasal cavity tumors and lung tumors were observed in all three species.

Increases in rare stomach tumor, rare esophageal tumors, rare glandular stomach tumors, liver hepatocellular adenoma/carcinoma and liver hemangioma/hemangiosarcoma were observed in two species, mice and rats.

Increases in rare liver cholangioma/cholangiocarcinoma, rare oropharyngeal tumors, and reticuloendothelium tumor were observed in mice. An increase in rare tongue tumors was observed in rats. Lastly, increases in rare laryngeal and tracheal tumors were observed in hamsters in both sexes and in two generation studies in both exposed dams and in the F1 offspring in both sexes.

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DR. HSIEH: Continuing on summary of other relevant data.

NHEX is bioactivated by cytochrome P450s to form a number of electrophilic and/or genotoxic metabolites, as summarized on this slide. NHEX has been tested for

genotoxicity and found to be mutagenic in bacteria in Chinese hamster lung V79 cells in vitro and in Drosophila in vivo. And in rats exposed in vivo, NHEX was found to bind covalently to liver RNA and DNA.

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DR. HSIEH: Finally, there are strong structure-activity similarity between NHEX and five comparison heterocyclic nitrosamines, four of which are listed as carcinogens under Proposition 65.

Several QSAR models predict that NHEX is both mutagenic and carcinogenic. And the mechanistic findings for NHEX are associated with two key characteristics of carcinogens, shown here: Can form electrophilic metabolites, and is genotoxic.

That concludes today's presentation.

Thank you.

CHAIRPERSON MACK: Thank you, Dr. Tsai and thank you Dr. Hsieh. Does anybody have any questions for the staff?

COMMITTEE MEMBER EASTMOND: I have a question. Do you have a -- you have a lot of place you indicate this is a rare tumor. How do you distinguish rare from uncommon, et cetera? How is that --

DR. TSAI: Okay. Generally speaking we use less than one percent in historical control for rare. And we

use this definition from the IARC pathology or any published paper. But uncommon is when sometimes in the pathology books or in some, for example, the New Zealand Inbred Mice, the authors would say this tumor is uncommon. So uncommon would be something around roughly one to two, three percent. Yeah.

COMMITTEE MEMBER EASTMOND: That's fine. No, I was just curious, because I was tying to figure it out.

DR. TSAI: For rare, we have more stringent standards. It has to be rare, not by our definition, but by the common accepted definition.

COMMITTEE MEMBER EASTMOND: Thanks.

DR. SANDY: And I'll just add, Dr. Eastmond that --

CHAIRPERSON MACK: Anybody else?

DR. SANDY: Yeah. Can I -- this is Martha Sandy. If I can just add in the pathology section of the documents, we do try to go tumor site by tumor site and give some citations. When we say that something is rare or uncommon, we're citing a paper or a reference that tells you that.

CHAIRPERSON MACK: Anybody else have a questions?

All right. Let's go to Joe.

COMMITTEE MEMBER LANDOLPH: This one is similar to the other one in that there's a lot of data here. And

I really liked the hazard identification document. It's fantastic. Keep doing them this way. It's great.

What impressed me first was that there were three species in which you had positives. And then the next thing I looked at was there were male and female, both were positive. And if I remember right, there were 10 assays in the mice, eight in the rats, and four in the hamsters. So that's a lot of data positive just on the tumorigenicity standpoint.

Then in addition to that, there the classical cytochrome P450 metabolism. Many of the metabolites are mutagenic and clastogenic. So that was great. Cytochrome P450 mediated production of metabolites, which are genotoxic.

And then I really thought that the data on the congeners was very helpful. So that was all positive, and many of these were carcinogens as well. And I think you mentioned that some of these metabolites were carcinogens on the Prop 65 list. So that's all very good.

So it fits together for me in a compelling set of convincing evidence, which is all consistent. So thank you. I think you did a great job. And I'm very satisfied with this one. I have no problem, in my opinion, stating that this is a chemical that has a lot of evidence that all points in the same direction, that of a significant

carcinogen.

CHAIRPERSON MACK: Thank you, Joe.

Jason.

COMMITTEE MEMBER BUSH: All right. Well, I, too, want to thank the OEHHA staff for those of you that contributed and reviewed this. I was really impressed by the scope and the extent of the literature search strategy that was indicated in the appendix for this chemical. And it really did give the impression that you left no stone unturned. So well done and keep doing it that way, please.

With respect to NHEX, like Joe said, I did find the weight of the evidence compelling. No human data, so we really are consigned to the other surrogate data, particularly the animal studies, finding 33 of these species specific studies.

I think it was great. The way it was outlined in the table was very helpful. It's clear that most of these were epithelial in nature when they affected the GI tract. And that is consistent with the direct exposure, either through drinking water or the gavage route.

The presence of liver tumors I think is consistent with the carcinogenicity of the metabolites. Likewise, with the other tumor types found in the exhalation pathways associated with CO2 excretion.

You did state in the HID that several of these studies were limited by small numbers of animals, lack of concurrent controls and limited duration of exposure. But these were some very old studies. And despite that, I think you did a great job kind of teasing out some statistically valid data from that. So thank you.

The positivity for the mutagenic outcomes in bacteria, in the mammalian cells in vitro in flies, again was for me compelling positive data. The significant DNA/RNA binding liver preparations from rats after in vivo exposure, again alluding to the metabolite connection here.

The structure activity considerations, we just saw the table, and I think again continuing compelling data for these cyclic nitrosamines that we have listed previously.

The tumor site comparisons, and particularly in table 13, and the genotoxicity comparisons in table 14 were really convincing as well.

I -- in terms of the ToxCast data, as my colleague Dr. Landolph said earlier, I think we have to be careful with that information. But it's still informative and it is good to see that. You did identify an increase in the pregnane X receptor, PXR receptor. It's a nuclear receptor. We know that this is involved with xenobiotic

metabolism of various compounds. We know that it's -- it interacts with CYP, so that all fits with the -- you know, the assumption of the mechanism, and is consistent with that -- the mechanistic evidence of that -- of electrophilic metabolites.

And finally, the fact that the European Chemicals Agency classifies it as a class 1B carcinogen, I was convinced.

CHAIRPERSON MACK: Thank you, Jason.

We'll go down the list again now. Shanaz, do you have any comments?

COMMITTEE MEMBER DAIRKEE: I don't have any additional comments.

CHAIRPERSON MACK: Tom.

COMMITTEE MEMBER McDONALD: First, I wanted to give some feedback that I absolutely love the table format that you presented. It made review of each study very good. I like the fact that you had all the species, strain information, the dose, and the regimen, survival, incidence -- including incidence and percentage all in one spot, made it really nice to review.

It's clear this is a model carcinogen, a transplacental carcinogen, and there's a very strong weight of the evidence. I just had one question what the heck happened with ToxCast? It seemed to be amiss.

(Laughter.)

COMMITTEE MEMBER McDONALD: If you guys can talk about that later. That's it.

CHAIRPERSON MACK: Michelle.

COMMITTEE MEMBER LA MERRILL: I have nothing additional to add.

CHAIRPERSON MACK: David.

COMMITTEE MEMBER EASTMOND: A couple things. First, a commentary on ToxCast. This is sort of the model example of when ToxCast failed. Okay. And it's largely because the screening assays used in ToxCast do not -- are not able to do metabolic activation properly. And this is a -- these nitrosamines require metabolic activation. And so it's basically a failure. So it was positive in 2 out of 276 assays, which is really surprisingly negative for this compound.

The other thing I might mention -- so, clearly this is consistent with other nitrosamines. It's a very potent carcinogen in many species. One thing I might point out to you is in -- something I believe is incorrect in your metabolism pathway. That one 1,6-hexanediol is not electrophilic and will not bind to DNA. If you go back to the original paper, that's the metabolite which is released after you do acid hydrolysis. So presumably either your -- the hexanol derivative or probably

carbonium ion is the one that's actually binding to the DNA or RNA in this case.

And then they treat it with concentrated hydrochloric acid, which releases the hexanediol. So I just the idea is that it's not the binding species. It's the species which is adducted to the DNA. Okay.

But apart from this, obviously -- this is a very strong positive and should be listed, in my opinion.

CHAIRPERSON MACK: All right. Mariana.

COMMITTEE MEMBER STERN: I don't have much to add. I just want to emphasize the point that Joe made that I think it's very compelling that four out five of the chemotypes are already in Prop 65 list, and they share the same tumor sites. So that makes it an even stronger carcinogen.

CHAIRPERSON MACK: Peggy.

COMMITTEE MEMBER REYNOLDS: I really don't have anything to add. I do want to mention that I actually thought it was helpful that you added some of the evidence from the European Chemicals Agency in their very recent assessment of this as a category 1B carcinogen was helpful as well.

CHAIRPERSON MACK: Luoping.

COMMITTEE MEMBER ZHANG: Yeah. Not much, but thank you. Thank the staff. And again it's a very good

presentation. So in comparison with the first chemical, it looks to me this one is a heavily, you know, focused on the animal study, but I just -- I have a question before -- one, another question is on the table. I noticed the carcinogenicity study, the summary three different species. And for the rats, you have experiment seven, but it -- then the rats bioassay, then come to six. So is that typo or is it some study?

DR. TSAI: No, it's not, because on the slide --

COMMITTEE MEMBER ZHANG: Okay. Can you explain it to me. I just --

DR. TSAI: Yeah. On the slide, we didn't include the subcutaneous injection study. That is one study that's reported in German abstract with very limited findings or information. So we excluded it from the study overview. But it is included in the table 2 or in the HID.

COMMITTEE MEMBER ZHANG: I see. Okay. So I thought maybe some -- one study excluded.

So to me, this one, like in both in mice and the rat studies, it's a single dose mostly. But still I think for the data, it's still multiple species and the multiple strains, and the multiple studies, and the both sex, even though lots of cancers in the rare cancer. So I still think it's pretty convincing.

But also I'm glad to see they have the hamster studies with really multiple dose. So if without -- if it was only -- you know, everything is only a single dose, you know, compared with control, I would be a little bit, you know, worried. So anyway. I think that's pretty good.

CHAIRPERSON MACK: Thank you. Does anybody have any final afterthoughts?

If not, is there anybody in the public who'd like to stand up and vote?

I guess not.

So then it's time for another vote.

So the question is where is my -- where is my cheat sheet?

There it is. There.

CHAIRPERSON MACK: Okay. Has n-nitrosohexamethyleneimine been clearly shown through scientifically valid testing, according to generally accepted principles to cause cancer?

All of those voting yes, please raise your hand.

(Hands raised.)

CHAIRPERSON MACK: Voting no?

(No hands raised.)

CHAIRPERSON MACK: Again, unanimous.

And we've decided to list this compound as well.

And now we move on to additional activities, the first of which is an update of section 25 -- 27000 regulations that list the chemicals requiring testing by the federal and the State.

So Carol, you're going to give a presentation.

(Thereupon an overhead presentation was presented as follows.)

CHIEF COUNSEL MONAHAN CUMMINGS: Right. Thank you, Dr. Mack. So this is a consent item for this Committee. We already provided you with a report earlier. Hopefully all of you had a chance to look at it. The report summarizes information received from relevant -- other authoritative bodies.

Let's see, so the staff report we sent you looks like this. I don't know if it's in your materials.

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CHIEF COUNSEL MONAHAN CUMMINGS: So essentially what we are recommending is -- let's see, let me back up here.

This is the section 2700[sic]list of chemicals that require additional testing for cancer reproductive toxicity endpoints. It's not the same list as the more well known Prop 65 list. So this one, we rely on U.S. EPA and the Department of Pesticide Regulation within CalEPA to give us information about mandatory testing

requirements for various chemicals.

So in your -- in the staff report, we gave you information about the chemicals that the Department of Pesticide Regulation has said have sufficient testing now and should be removed from the section 2700[sic]. Those are here. The only I can pronounce is Borax.

(Laughter.)

CHIEF COUNSEL MONAHAN CUMMINGS: But you can see on the -- on the slide -- this is why I went into law and not science.

(Laughter.)

CHIEF COUNSEL MONAHAN CUMMINGS: Next -- well, that's me. Next slide.

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CHIEF COUNSEL MONAHAN CUMMINGS: All right. So the same here, but this is as reported by U.S. EPA, there's five chemicals that they have recommended or that there's -- testing is fully satisfied and should be removed from our section 2700[sic] list. Those are here on this slide.

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CHIEF COUNSEL MONAHAN CUMMINGS: And then there's some additions that are recommended by the Department of Pesticide Regulation. And these are specific tests for sodium fluoride. And so we're recommending that we add

those -- those testing endpoints.

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CHIEF COUNSEL MONAHAN CUMMINGS: And the same here for these two -- well, one chemical, and a class of chemicals. These are recommended by DPR as still needing certain testing. And so you can see those here. The strikeout and underline are the things that we're adding on this particular item.

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CHIEF COUNSEL MONAHAN CUMMINGS: And on this slide, you can see those chemicals the Department of Pesticide Regulation believes need to be added -- additional endpoints for testing to the section 2700[sic] list.

All right. So what we're asking this Committee to do is since this is consent, would you consent to our office adding and deleting the chemicals and endpoints that need testing that were recommended by U.S. EPA and DPR that are described in the staff report?

CHAIRPERSON MACK: Thank you, Carol.

Does anybody on the Committee have any specific questions about the individual items or about the general consent procedure?

It seems not.

So again, we have a standard question. Based on

the recommendations of the OEHHA staff report, should section 27000 of Title 27 in the California Code of Regulations be amended, as indicated in section 6 of the staff report? All those voting yes, please raise your hand.

(Hands raised.)

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

(No hands raised.)

CHAIRPERSON MACK: And all of those abstaining.

(No hands raised.)

CHAIRPERSON MACK: So we unanimously agree to amend the section 27000 as indicated.

CHIEF COUNSEL MONAHAN CUMMINGS: Thank you.

CHAIRPERSON MACK: Staff updates.

Julian.

MR. LEICHTY: Okay. Thanks. Since your last meeting, we have added -- oh, thank you -- we've added five chemicals to the list for the -- okay. Since your last meeting, we have added five chemicals to the list for the endpoints as shown, chlorpyrifos, n-hexane, vinylidene chloride, TRIM VX and nickel (soluble compounds).

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MR. LEICHTY: There are two chemicals under consideration for administrative listing or modification

of existing listing. A notice of intent to modify the listing of ethanol in alcoholic beverages was published on August 3rd, 2018. This is proposed under the Labor Code listing mechanism for the cancer endpoint.

A notice of intent to list bevacizumab was published on October 5th, 2018. It is under consideration for administrative listing under the formally required mechanism for the female reproductive and developmental endpoints.

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MR. LEICHTY: Here you'll see the four safe harbor levels we've adopted in regulation since your last meeting. For malathion, a no significant risk level of 180 micrograms per day effective April 1st, 2018.

For glyphosate, a no significant risk level of 1100 micrograms per day adopted effective July 1sst, 2018. For Vinylidene chloride, a no significant risk level of 0.88 micrograms per day adopted effective July 1st, 2018. And for metham sodium, a maximum allowable dose level of 290 micrograms per day adopted effective October 1st, 2018.

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MR. LEICHTY: And finally, you'll see we proposed safe harbor levels for three chemicals. No significant risk levels for bromochloroacetic acid, and

bromodichloracetic acid. And maximum allowable dose levels for n-hexane by the oral and inhalation routes.

COMMITTEE MEMBER EASTMOND: Can I ask a question?

CHAIRPERSON MACK: Thank you, Julian.

COMMITTEE MEMBER EASTMOND: Tom, can I ask a question?

CHAIRPERSON MACK: Oh, David.

COMMITTEE MEMBER EASTMOND: So I take it that they've struck ethanol out of the ethanol in alcoholic beverages. The proposal was to eliminate that. Is there a reason for that? I mean, I thought the -- that's --

MR. LEICHTY: I'll defer to Carol.

CHIEF COUNSEL MONAHAN CUMMINGS: Yeah. So that's a proposal right now that we have made to modify one of the listings of alcohol under alcoholic beverages under Prop 65. There's at least three other ones. So this is based on the IARC -- a fairly recent monograph from IARC, along with a couple of older monographs where they initially had identified ethanol in alcoholic beverages as causing cancer. And now they're just saying, as a general rule, alcoholic beverages cause cancer.

So, you know, probably still the primary is ethanol, but there are other chemicals in alcoholic beverages that probably contribute to cancer. So it's really more of a kind of a ministerial change to be

consistent with IARC.

COMMITTEE MEMBER EASTMOND: Okay. I mean, because if anything, the most recent IARC review for me emphasized that the ethanol was playing a critical role through acid aldehyde. That was it, so...

CHIEF COUNSEL MONAHAN CUMMINGS: Um-hmm, right.

CHAIRPERSON MACK: Now

DIRECTOR ZEISE: Martha is going to --

DR. SANDY: And I'll just add that there also are many other things in alcoholic beverages that are carcinogens.

COMMITTEE MEMBER EASTMOND: Including nitrosamines that we've been talking about.

(Laughter.)

CHAIRPERSON MACK: Carol, do you want to tell us what danger we're in?

(Laughter.)

CHIEF COUNSEL MONAHAN CUMMINGS: Oh, sure. Yeah, I think that actually it's OEHHA that's in the most danger at the moment.

But this is the litigation update since your last meeting. We had a State court case that had been filed against the office by Monsanto, among others, regarding our listing of glyphosate as a carcinogen under Prop 65. That case has been resolved now in OEHHA's favor. Both

the trial court and the court of appeal agreed that the chemical was properly listed, and the California Supreme Court declined to take the case for review.

A related case is currently pending in the federal trial court. That's called National Association of Wheat Growers versus Dr. Zeise. And it's also related to glyphosate. What's unusual about this, there's two things. One is that we're in federal court. This is, as far as I know, the first time a case has been filed against OEHHA and the Attorney General's office in federal court over Prop 65.

The reason that it's in federal court is that the primary basis for the challenge is to the warnings for -- potential warnings for glyphosate. And the argument is that those would violate the First Amendment rights of the corporations and individuals that would have to give the warning.

So currently, the federal court has granted a motion for a stay of enforcement of the warning requirement. That stay -- or that order is actually only effective as to the Attorney General's office, because as you may know, we don't enforce Prop 65. So actually Dr. Zeise and our office have been dismissed from that case.

It is still pending in the federal court, but it has been stayed waiting for a couple -- actually, I think

there's three now Ninth Circuit cases that deal with First Amendment arguments in warning type regulations or statutes. So until those cases are resolved by the Ninth Circuit, the trial court in this case is not going to proceed.

So back to the State courts. We have several cases that are still on appeal. The American Chemistry Council case against OEHHA regarding the listing of BPA is still pending. It's been in the Court of Appeal since 2015. We're still waiting for a hearing date. It's been fully briefed.

The second case against OEHHA by the American Chemistry Council has to do with the listing of DINP by this Committee, I believe.

DR. SANDY: Yes.

CHIEF COUNSEL MONAHAN CUMMINGS: And that one is also still pending in the court of appeals since 2016. It's fully briefed and we are waiting for a hearing date. As you may know, the Courts of Appeal have to take criminal cases first. They have limited resources, so the civil cases tend to get pushed back.

Then the other case that's still pending in the Court of Appeal is one filed by Syngenta company against OEHHA for the listing of a group of a triazine pesticides. That case is in the appeal court fully briefed waiting for

a hearing date.

There's two derivative cases to those that are in the State court, but are not active, and that has to do with PRA requests that are related to those two cases.

The newest case that we have was filed in September of 2018. It was filed by an enforcement group called CERT Center for Research on Toxics, and you may have heard about that since you all received comments from individuals on -- that are involved in that case. It has to do with a proposed regulation that OEHHA has pending on whether or not the -- whether coffee is -- causes a significant risk of cancer.

We were sued in State court in Los Angeles. And that is very much at the beginning stages of litigation. We were just recently assigned to a new judge and will be starting to hear motions and things like that starting November the 21st.

So just as a reminder, there is a litigation hold on your -- any documents you have or communications with our office related to that case. And I can talk to you offline if you have questions about that.

Any questions about these?

CHAIRPERSON MACK: David.

COMMITTEE MEMBER EASTMOND: Can I make a request? Can you send out an email to us indicating the chemicals

that we need to be holding on to, these litigation holds? Because I start forgetting them. You know, I got this stuff piling up, and I like to throw stuff away. And I don't remember which ones. You know, there's enough of them now that it's kind of hard to keep track of it. So if you --

CHIEF COUNSEL MONAHAN CUMMINGS: Okay.

COMMITTEE MEMBER EASTMOND: -- could just kind of say at least these are the ones you need to worry about and --

CHIEF COUNSEL MONAHAN CUMMINGS: Sure. Off the top of my head, I think you only have two for this Committee, but I could be wrong. Some of them have been released because the cases have been resolved, so make sure that you know that.

COMMITTEE MEMBER EASTMOND: Okay. That's useful to know.

(Laughter.)

CHIEF COUNSEL MONAHAN CUMMINGS: Yeah, I'll follow up.

COMMITTEE MEMBER EASTMOND: It's better than throwing away stuff.

CHIEF COUNSEL MONAHAN CUMMINGS: Yes, please.

CHAIRPERSON MACK: Thank you, Carol.

And I guess that concludes our business for the

day. Lauren.

DIRECTOR ZEISE: Okay. Thank you. I'll summarize the Committee's actions for today. So the Committee voted that gentian violet and n-nitrosohexamethyleneimine were clearly shown through scientifically valid testing, according to generally accepted principles to cause to cause cancer.

And the Committee also amended -- recommended -- let's see based on the recommended -- recommendations in the OEHHA staff report that section 2700[sic] of Title 27 in the California Code of Regulations be amended.

So that was a summary of the Committee's actions.

And I guess in closing, I'd just like to thank the Committee so much for all of the time that you've spent preparing for this meeting, for coming to the meeting. It's all very much appreciated. So thank you.

And I'd also like to thank the staff for all of the hard work they did to pull all of the information together, their presentations, their hazard identification work, all the preparation for the meeting and all the preparation from the implementation staff and legal staff. So again all very much appreciated.

And finally, I'd just like to thank also those in the audience present and listening on the web for your participation in our Proposition 65 CIC activities.

So thank you very much one and all. Safe travels. And I'll turn it back over to Dr. Mack to adjourn the meeting.

CHAIRPERSON MACK: Feliz año[sic] de la muerte. It's the day of the dead. But with that, I'll just complete the meeting and let's call it a day.

(Thereupon the Carcinogen Identification Committee adjourned at 12:36 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 12th day of November, 2017.

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