MEETING

STATE OF CALIFORNIA

OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

PROPOSITION 65

DEVELOPMENTAL AND REPRODUCTIVE TOXICANT

IDENTIFICATION COMMITTEE

JOE SERNA JR.

CALEPA HEADQUARTERS BUILDING

1001 I STREET

SIERRA HEARING ROOM

SACRAMENTO, CALIFORNIA

WEDNESDAY, NOVEMBER 29, 2017

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A P P E A R A N C E S

COMMITTEE MEMBERS:

Ellen B. Gold, Ph.D., Chairperson

Patrick Allard, Ph.D.

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Suzan Carmichael, Ph.D.

Ulrike Luderer, Ph.D., M.P.H.

Aydin Nazmi, Ph.D.

Isaac Pessah, Ph.D.

Charles Plopper, Ph.D.

Tracey Woodruff, Ph.D., M.P.H.

STAFF:

Dr. Lauren Zeise, Acting Director

Mr. Alan Hirsch, Chief Deputy Director

Ms. Carol Monahan Cummings, Chief Counsel

Mr. Carl DeNigris, Staff Counsel

Dr. James Donald, Chief, Reproductive Toxicology and Epidemiology Section

Dr. Poorni Iyer, Reproductive and Cancer Hazard Assessment Branch

Dr. Farla Kaufman, Reproductive and Cancer Hazard Assessment Branch

Dr. Francisco Moran, Reproductive and Cancer Hazard Assessment Branch

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

STAFF:

Ms. Michelle Ramirez, Environmental Scientist, Proposition 65 Implementation Program

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

ALSO PRESENT:

Ms. Josephine Alvarado, Campesinas Unidas Del Valle De San Joaquin
Dr. Carol Burns, Dow AgroSciences

Ms. Lucia Calderon, Safe Ag Safe Schools

Mr. Benny Corona, Coalition Advocating for Pesticide Safety

Ms. Carol Erickson, Safe Ag Safe Schools

Ms. Katley Falconer, Coalition Advocating for Pesticide Safety

Dr. Katherine Foster, Physicians for Social Responsibility, American Academy of Pediatrics

Mr. Angel Garcia, Coalition Advocating for Pesticide Safety

Mr. Raul Garcia, Coalition Advocating for Pesticide Safety

Ms. Sandra Garcia, Campesinas Unidas Del Valle De San Joaquin

Dr. Kim Harley, University of California, Berkeley

Dr. Dalan Juberg, Dow AgroSciences

Ms. Kathleen Kilpatrick, Safe Ag Safe Schools

Mr. Stanley Landfair, Dow AgroSciences

Dr. Ann Lopez, Center for Farmworker Families

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

ALSO PRESENT:

Dr. Emily Marquez, Pesticide Action Network

Dr. Jay Murray, National Oilseed Processors Association, Institute of Shortening and Edible Oils, Grocery Manufacturers Association

Mr. Woody Rehanek, Safe Ag Safe Schools

Ms. Miriam Rotkin-Ellman, Natural Resources Defense Council

I N D E X

P R O C E E D I N G S

DIRECTOR ZEISE: Good morning, everyone. Hello. I'm Lauren Zeise. I'm director of the Office of Environmental Health Hazard Assessment. I'd like to welcome you all to this meeting of the Development and Reproductive Toxicant Identification Committee. We have two main things on the agenda. The consideration of chlorpyrifos and the consideration of n-hexane for potential listing under Proposition 65. We also have a consent item and some staff updates.

So before we move towards the Committee business, I'd like to go over a few logistics and also introduce the Panel and staff.

So first, simple logistics. Drinking fountains and restrooms are located out the back door and to the left end of the hall. You just go out the back, turn to the left, and they're located on the right side. In the event of a fire alarm, or any reason to evacuate this room, please leave by the lighted exits, and then take the steps down this -- down -- go down the stairs and then go outside and we'll locate across the street in the park.

So this meeting is being transcribed. It's also being translated into Spanish for Spanish speakers in the audience. And it's also being webcast. So please, everyone, speak clearly into the microphones and give your

name for the record.

I believe we're also going to have -- yes, we do have a interpreter, an American sign language interpreter here to my right. We'll also be taking breaks during the meeting for the court reporter and for the interpreters.

Okay. So now, I'd like to introduce the Committee, the Development and Reproductive Toxicant Identification Committee, which we'll periodically refer to as the DARTIC.

So just starting at the far end here, we have Dr. Aydin Nazmi from the California Polytechnic State University, San Luis Obispo; Dr. Suzan Carmichael, Stanford University School of Medicine. A new Committee member, who will be shortly sworn in, Dr. Patrick Allard, UCLA's School of Public Health. Next to him Dr. Ulrike Luderer, UC Irvine, School of Public Health. Then our Chair, Dr. Ellen Gold, UC Davis School of Medicine. Next to me, to my left, is Dr. Isaac Pessah, UC Davis School of Veterinary Medicine. Next to him Dr. Charles Plopper, UC Davis School of Veterinary Medicine. And then Diane -- Dr. Diana Auyeng-Kim, Genentech.

Okay. Great. So welcome, Committee.

Now, I'll turn to the OEHHA staff. So seated in the front at this long table we have Dr. Allan Hirsch, our Chief Deputy Director; next to him Carl DeNegris, staff

counsel; then Carol Monahan-Cummings, Chief Counsel; Martha Sandy, Branch Chief of the Reproductive and Cancer Hazard Assessment Section, or RCHAB; next to her, staff toxicologist Poorni -- Dr. Poorni Iyer; next to her, Dr. Farla Kaufman, staff toxicologist; and then Dr. James Donald, who is Chief of the Reproductive Toxicology and Epidemiology Section.

Then our Proposition 65 Implementation staff. And maybe if you could stand and wave, so people know who to give the cards to for speaking. We have Esther Barajas-Ochoa, and Michelle Ramirez, and then also Julian Leichty. And then in the audience Sam Delson, our Deputy Director for External Affairs. So welcome, everyone. And with that, now we'll turn to give the oath of office to the Patrick -- Dr. Patrick Allard.

So if you'd like to stand up, Dr. Allard. Okay. So if you'd hold up your right hand.

Is your mic on?

(Laughter.)

DIRECTOR ZEISE: Okay. Very good.

Okay. "I --

COMMITTEE MEMBER ALLARD: I --

DIRECTOR ZEISE: -- state your name --

COMMITTEE MEMBER ALLARD: -- Patrick Allard --

DIRECTOR ZEISE: -- "do solemnly swear" --

COMMITTEE MEMBER ALLARD: -- do solemnly swear --
DIRECTOR ZEISE: -- "that I will support and defend" --
COMMITTEE MEMBER ALLARD: -- that I will support and defend --
DIRECTOR ZEISE: -- "the Constitution of the United States" --
COMMITTEE MEMBER ALLARD: -- the Constitution of the United States --
DIRECTOR ZEISE: -- "and the Constitution of the State of California" --
COMMITTEE MEMBER ALLARD: -- and the Constitution of the State of California --
DIRECTOR ZEISE: -- "against all enemies" --
COMMITTEE MEMBER ALLARD: -- against all enemies --
DIRECTOR ZEISE: -- "foreign and domestic" --
COMMITTEE MEMBER ALLARD: -- foreign and domestic --
DIRECTOR ZEISE: -- "that I will bear true faith and allegiance" --
COMMITTEE MEMBER ALLARD: -- that I will bear true faith and allegiance --
DIRECTOR ZEISE: -- "to the Constitution of the United States" --

COMMITTEE MEMBER ALLARD: -- to the Constitution of the United States --

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

COMMITTEE MEMBER ALLARD: -- and the Constitution of the State of California --

DIRECTOR ZEISE: -- "that I take this obligation freely" --

COMMITTEE MEMBER ALLARD: -- that I take this obligation freely --

DIRECTOR ZEISE: -- "without any mental reservation" --

COMMITTEE MEMBER ALLARD: -- without any mental reservation --

DIRECTOR ZEISE: -- "or purpose of evasion" --

COMMITTEE MEMBER ALLARD: -- or purpose of evasion --

DIRECTOR ZEISE: -- "and that I will well and faithfully" --

COMMITTEE MEMBER ALLARD: -- and that I will well and faithfully --

DIRECTOR ZEISE: -- "discharge the duties" --

COMMITTEE MEMBER ALLARD: -- discharge the duties

DIRECTOR ZEISE: -- "upon which I am about to enter".

COMMITTEE MEMBER ALLARD: -- about which I'm about to enter.

DIRECTOR ZEISE: "Upon which" --

COMMITTEE MEMBER ALLARD: -- upon which I'm about to enter.

DIRECTOR ZEISE: -- "I'm about to enter".

Okay. Congratulations and welcome to the DARTIC.

COMMITTEE MEMBER ALLARD: Thank you.

(Applause.)

DIRECTOR ZEISE: Okay. And now, Carol Monahan-Cummings will give some introductory comments.

CHIEF COUNSEL MONAHAN CUMMINGS: Good morning. I just wanted to remind the Committee of a few items. I know that you've heard these before, except for maybe Dr. Allard. But since we only meet once a year, so I try to do these reminders for each meeting.

First, I'd like to remind you that in your binders, and in the materials that we provided you earlier, there is criteria -- scientific criteria that was developed by an earlier iteration of this Committee for listing chemicals under Proposition 65.

If you have questions about the data that you're looking at for a particular chemical, please refer to the criteria, which are in the back of the binder, that you were given today under the tab "Criteria". Those are

scientific criteria that were developed by the Committee. And the intent of those is to provide guidance. There's lots of room for judgment calls in the criteria for good reason.

Obviously, science moves forward, and the application of the criteria has to move with the science. And so hopefully that criteria is useful to you.

The charge for this Committee has to do with listing chemicals under Proposition 65. Sometimes, through some of the comments that you hear, you'll be told other information that has to do with the impact of a particular listing, for example, whether or not a warning is or might be required for that chemical, or particular impacts on certain sectors of the economy.

While that information is helpful in a general sense, it isn't part of the criteria for this Committee, and so you should apply the criteria that you have available in your binders, in addition to applying your own scientific judgment on the questions that are put before you.

You'll also hear about the clearly-shown standard, which is part of the statute. You're required to find whether or not a chemical has been clearly shown through scientifically valid testing, according to generally accepted principles to cause developmental

toxicity or reproductive toxicity. This is a scientific question, and is not a legal standard of proof.

This Committee is also allowed and often does make decisions based entirely on animal evidence. The chemicals that you are considering do not need to have been shown to be human reproductive toxicants. You don't need to have information about whether or not human exposures to the chemicals are sufficiently high enough to cause reproductive toxicity or developmental toxicity in order to list the chemical.

The members of this Committee are very well qualified scientists. You were appointed to the Committee by the Governor, because of your scientific expertise. And you don't need to feel compelled to go outside that charge and make other kinds of decisions.

In the event that you have, or you feel you have, insufficient information or questions that need to be responded to, or you need more time to think or discuss the questions that are before you, there is no requirement that you make a decision today on any of the questions that will be presented. You can always ask the staff to respond to a question, or prepare additional information, and you can ask to defer the question to another meeting.

Does anybody have any questions on that?

Thank you.

DIRECTOR ZEISE: Thank you, Carol.

Okay. Now, I'll turn the meeting over to Dr. Gold.

CHAIRPERSON GOLD: Thank you. Good morning. Before we begin, I want to say something about public comments. We'll get to those a little bit later, but if -- but our usual process is that each speaker has five minutes, except for those that have made a request by October 30th for a different amount of time for longer comments. There are blue cards available in the back on the back table. So if you wish to make a comment, please fill out the card and return it either to Esther or Michelle.

Also, before we begin, I want to make a disclosure. So I participated in the U.S. EPA's 2012 Scientific Advisory Panel review of chlorpyrifos. I was on the panel and was the lead discussant on the epidemiologic studies regarding child health described in the 2014 EPA risk assessment document, and provided responses to the charge questions posed by EPA.

We also discussed the responses in a public meeting of the Scientific Advisory Panel. We did not recommend any regulatory actions for EPA to take regarding chlorpyrifos.

And Dr. Pessah also has a disclosure.

COMMITTEE MEMBER PESSAH: Yes. I wish to disclose my participation in the 2016 EPA FIFRA Advisory Panel. The Panel was convened to advise the U.S. EPA regarding the evaluation of biomonitoring data on chlorpyrifos from a epidemiological studies. I participated as a member of the Scientific Review Board. We did not recommend any specific regulatory actions for EPA to take regarding chlorpyrifos.

CHAIRPERSON GOLD: Thank you.

So at this point, I'll turn it over to Dr. Sandy for a staff presentation.

(Thereupon an overhead presentation was presented as follows.)

DR. SANDY: Thank you very much and good morning to everyone.

My name is Martha Sandy, and I will provide you with a bit of background on chlorpyrifos, the first chemical you'll be considering today.

So nine years ago in 2008, chlorpyrifos was considered, but not listed by this Committee. Since that time, many studies have been published on chlorpyrifos, and a great many of those have been focused on developmental toxicity. Today, you are considering whether chlorpyrifos should be listed as known to cause reproductive toxicity based on the developmental toxicity

endpoint.

I will now turn this over to Dr. Jim Donald who will provide a brief overview of the hazard identification materials provided to this Committee.

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DR. DONALD: Thank you, and good morning.

Okay. As you've just heard, chlorpyrifos was previously considered by this Committee in 2008. And since that time, substantial new epidemiological and toxicological data on chlorpyrifos have become available, particularly in the area of neurobehavioral developmental toxicity.

Because of the volume and complexity of those data, your being asked today only to consider the developmental endpoint, but the other relevant endpoints, such as male or female reproductive toxicity may be considered by this Committee at future meetings.

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DR. DONALD: In terms of the materials provided to you for this meeting, consistent with our usual practice when there is a recent comprehensive review of toxicity of a chemical prepared by another body, we provided that to you in lieu of OEHHA developing its own hazard identification document. So we provided you with two iterations of the U.S. EPA Human Health Risk

Assessment. The revised version is published in 2014 and 2016. And these reports, in particular the 2014 report, extensively review the relevant scientific literature on chlorpyrifos and developmental toxicity. Those documents also covered other areas of toxicity. So for the Committee's convenience, we exerpted the sections of the reports that are relevant to developmental toxicity and provided those to you.

We also provided you with copies of the studies relating to developmental toxicity of chlorpyrifos that were cited in the U.S. EPA report, so practically all of the studies that were cited in those excerpted sections.

OEHHA also conducted its own additional literature searches for additional information on the developmental toxicity of chlorpyrifos that were not reviewed in the 2014 or 2016 U.S. EPA reports, or in the materials that the DARTIC had reviewed in 2008. And we provide you with copies of all of the relevant studies that we identified.

And finally, again, consistent with our usual practice, these materials were released for public comment. And all of the comments received were provided to the Committee.

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DR. DONALD: We also provided you with all of the

materials that the Committee had reviewed in 2008, including the public comments that were received on those materials at that time. And again, for the Committee's convenience, we excerpted the sections of our 2008 hazard identification document that dealt with developmental toxicity.

We also provided you with copies of the studies that relate to developmental toxicity that were cited in that 2008 hazard identification document.

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DR. DONALD: So, in total, the scope of the information before you today is comprised of 390 papers or reports relevant to developmental toxicity of chlorpyrifos. Three hundred and seventeen of those reports were reviewed either by U.S. EPA in the 2014 or 2016 documents, or by OEHHA in 2008, or in some cases by both groups.

We also provided you with an additional 73 reports that were not cited in any of those three review documents. Most of those were published subsequent to the 2014 U.S. EPA report -- excuse me, review.

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DR. DONALD: So as we said, there's a substantial amount of additional information on chlorpyrifos since the last time the Committee looked at this chemical. The 390

papers include a great number of papers that provide direct empirical evidence on developmental toxicity of chlorpyrifos, but they also include a number of other studies on other related areas, such as potential mechanisms of action, and human exposures, and so forth.

We have identified at least 81 additional publications since 2008 that provide direct empirical evidence on developmental toxicity of chlorpyrifos and provided those to the Committee. Eighteen of those were reported on epidemiologic studies in humans, and 63 reported on experimental studies in animals.

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DR. DONALD: And finally, the additional studies that were not reviewed by OEHHA or by U.S. EPA are comprised of three human studies, two of which looked at neurodevelopmental endpoints, 25 studies and other mammalian species, 19 of which looked at neurodevelopmental endpoints. And I'll take this opportunity to just remind the Committee that as discussed in the 2008 hazard identification document, the early postnatal period and common rodent models, such as rat and mouse, are developmentally equivalent to pre -- the prenatal developmental period in humans. And therefore data from post-natal exposures up to at least day 10 and possibly a little later in rats and mice are relevant to

your deliberations today.

We also provided you with 14 studies, and -- using the relatively new -- or relatively recently developed zebrafish model; fifteen studies that looked at potential mechanisms of action for neurodevelopmental toxicity following in vivo exposures in animals; and 21 other papers covering a variety of related topics such as in vitro mechanistic studies on enzymes and paraoxonases and so forth.

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DR. DONALD: So I will stop there, and I'll be happy to answer any questions you have.

CHAIRPERSON GOLD: Do any Committee members have any questions of the staff?

CHAIRPERSON GOLD: Seeing none, we will move on to Committee discussion, and we'll start with -- we're going to start with animal studies of neurobehavior and neurodevelopment. And the first discussant is Dr. Pessah, who has a presentation, I believe.

COMMITTEE MEMBER PESSAH: Eventually, we'll get to that. I'll present a couple slides.

CHAIRPERSON GOLD: Okay. You'll clarify for us. Okay.

COMMITTEE MEMBER PESSAH: Good morning, and thank you. I was asked to review the animal behavior

literature, which involves several hundred papers, on, in particular, developmental neurotoxicity or evident scientifically sound evidence for developmental neurotoxicity.

Since I'm also the first speaker, I felt like I need to put the issue in context of some of the areas that other speakers will be presenting. So first a few facts about chlorpyrifos. It's a broad spectrum insecticide, meaning that it really targets many different species with application. It's actually the most highly used chemotype in agricultural and industrial professional pest control.

In a recent review from Casida and Bryan published in 2017 listed as the number one insecticide, single insecticide used in the world with 46,500 metric tons used annually, which translates into about 102 and a half million pounds per year, at a sales of about half a billion.

In the California Peer Report in 2015, in California particularly, there was about four and a half million pounds used. And that makes -- of insecticides, and of which 1.1 million pounds were chlorpyrifos, about 25 percent. So chlorpyrifos use is predicted by the Grand View Research to increase through 2022. So it is an environmentally relevant compound.

So in assessing behavioral consequences of

chlorpyrifos, one has to address not only the behavioral outcomes, but also biological plausibility that produce these behavioral outcomes. And I'll try to hit on some of the major points that need to be addressed as one goes through AOP or adverse outcome pathway.

Once you identify the chemical in this case, it's chlorpyrifos, but also its metabolite, chlorpyrifos oxon, you have to ask what are the levels of exposure and what level should you target in animal studies?

What are the molecular mechanisms in response? Well, chlorpyrifos is an organothionate, which has to be metabolized to an oxon in order to inhibit its primary target acetylcholinesterase. So one needs to accommodate both for metabolic activation, but also metabolic inactivation of the active material.

One needs to address what are the tissue responses and how do they relate to possible in vivo outcomes. Is there frank neuropathology or is it so much more subtle that a pathologist won't pick up on biological responses as they relate to changes, let's say, in the neuronal network organization.

Of course, we have to define clearly -- we have to clearly define health outcomes. And then we also need to account for genetic susceptibility, either at the metabolic level or at the end target.

So why is chlorpyrifos relevant and what makes it different from other organophosphates?

As I mentioned, it's a phosphorothionate with two ethyl esters, the third ester is what makes it special. It's a trichloropyridinol group, which means that it's a halogenated organic, which contains a 6-membered ring, 5 rings -- 5 members of that ring are carbon, 1 is nitrogen. So the thionate does not inhibit acetylcholinesterase. But once it's metabolized to the oxon, it's a potent inhibitor of acetylcholinesterase. And I'll get to how potent that is in a second.

But the outcome if you talk about lethal toxicity in the short-term exposure, that ranges quite a bit from species to species. The lowest I could find in the literature was around 5 milligrams per kilogram, which makes it extremely toxic. This is in certain species of wild birds to about 100 to 200 milligrams per kilogram in lab animals, such as rats and mice to some resistant organisms, where the toxicity is greater than about 1000 mg/kg this includes the rabbit.

These differences are likely due to different levels of carboxylesterases in the blood, which serve as a sink for the active principal, binds it up, and keeps it from targets that are relevant.

There are additional detoxifying mechanisms, such

as PON1, which I'm sure you'll hear about later, which metabolize chlorpyrifos to a DAP, a dialkyl phosphate, and TCP, which is 2,3,5-trichloropyridine. The dialkyl phosphates are not specific to just chlorpyrifos. Virtually every other organophosphate that's metabolized generates dialkylphosphate. So if you measure dialkylphosphates you're not measuring chlorpyrifos alone. You're measuring the aggregate of all organophosphate that the animal or the individual has been exposed to.

What makes chlorpyrifos also unique relevant to the other 12 major organophosphates used, the top 12, is that it has a log P, a lipid water partition coefficient, of 5, which makes it much more lipophilic than 10 of the other top organophosphates. What does that mean?

It means that it can distribute into fat, it can distribute across the blood-brain barrier, and it can get to the brain and have a distribution between lipid compartments and actual target sites, which are proteins.

So in terms of exposure, human exposures have been identified. And I'm just going to touch on this. But a study from UC Berkeley, the CHAMACOS study, identified 70 to 80 to 90 percent of individuals measured either at the maternal side or the cord blood side have detectable levels, measurable levels of chlorpyrifos in those samples.

California -- Californians are exposed, in general, to chlorpyrifos. And this seems to be correlated to proximity to application sites, as shown by Dr. Hertz-Picciotto in the CHARGE Study.

Chlorpyrifos can be found in breast milk. A study published by UC Berkeley in the Journal of Environmental Monitoring in 2011 indicated 90 percent of urban and ag workers in California have detectable levels. So it's sufficiently lipophilic to get into various compartments of concern when you talk about developmental neurotoxicity.

Does CPF cross the blood-brain barrier in animals. There are several studies primarily from the ARIC lab, published in a series of papers, that clearly show that CPF, as I'll call it for short, not only can cross the blood-brain barrier, but interacts with the blood-brain barrier both by incorporating into those cells that make up the blood-brain barrier, but also changes the resistivity, or the permeability of the blood-brain barrier at relatively low concentrations, concentrations that are in the neighborhood of 1 micromolar.

Chronic exposure during the perinatal period does, in fact, alter these tight junctions that form that permeability barrier in the BBB, the blood-brain barrier, and targets have been identified. Molecular targets have

been identified within those tight junctions.

So in my review of the literature, I tried to find whether there was any evidence that chlorpyrifos actually is in embryonic brain samples. Obviously, a very difficult study to do, if not impossible. But there are two pieces of information that can be gleaned from the human literature, the clinical literature where postmortem brains were harvested from SID individuals. These are fetuses that have undergone sudden intra -- intrauterine unexplained death syndrome, SIUD, or sudden infant death syndrome, SIDS, which clearly showed measurable levels of chlorpyrifos in the brain of those fetuses. Not all of them, but a fraction of them that was statistically defensible.

One of those papers I have to admit I actually couldn't find the data. It was actually the text portion in Frontiers of Neurology, but I imagine it underwent peer review.

The next question that I tried to address in animal studies is this CPF cross-placental barriers during gestation and does it alter the integrity of the placental barrier. Ridano just published a paper in Toxicology and Applied Pharmacology on the impact of chlorpyrifos on human villous trophoblasts and chorionic villi. They evaluated the effects of CPF on human placenta using in

vitro methods and ex vivo exposure animal models.

Basically, what they found that was chlorpyrifos in the neighborhood of 10 to 100 micromolar increases the expression of key barrier proteins that are involved in shuttling chlorpyrifos out of the fetus, in other words, protective mechanisms. And it turns out that one of those transporters is, what we call, an ATP-binding transporter, for short ABCG2.

And it turns out that chlorpyrifos is a substrate for ABCG2. So in other words, it can bind to that transporter and the transporter shuttles it out. Okay. So that would be considered a protective mechanism.

However, it turns out that studies unrelated to chlorpyrifos have shown that there's a high level of polymorphism in ABCG2, which, in fact, inactivates that transporter. And so one has to now think that although there are these defensive mechanism at the placental barrier, that there are polymorphisms in the human population which impact the efficiency of that transport mechanism. So that needs to be considered as we review both animal and human studies.

So what are the molecular targets of chlorpyrifos? Acetylcholinesterase inhibition is a very active catalytic enzyme, primarily present at virtually all nicotinic and muscarinic synapses.

Why is that important?

Well, it turns out that nicotinic and muscarinic synapses are key to neurotransmission, both in the developing nervous system, as well as early postnatally and obviously throughout life in terms of potential acute effects. But in particular, acetylcholinesterase breaks down acetylcholine at all central cholinergic locations. And so an imbalance in cholinergic signaling will have an impact on the level of excitability in the central nervous system.

And we know that during development, the excitability of the nervous system really dictates neural network connectivity. So one needs to keep that in mind. It may be short of producing measurable histopathological lesions that can be seen under light microscope, but certainly what can be seen with more sophisticated techniques that actually measure network connectivity and network morphometry.

So let me go on to another mechanism that's been proposed for chlorpyrifos and is related to acetylcholinesterase is that acetylcholinesterase, in addition to its catalytic function, also has a morphogenic function. That is, you don't need to hydrolyze acetylcholine with acetylcholinesterase for acetylcholinesterase to influence the growth and

development of neurons during development. It has a morphogenic function. And the work from the Lien Lab initially at Johns Hopkins in a series of papers and then her tenure at OHSU, and currently at UC Davis clearly shows that acetylcholinesterase has morphogenic functions, both in the peripheral nervous system, but also the central nervous system, and that chlorpyrifos can influence those morphogenic functions at relatively low levels.

And possibly these are the most potent effects that have been measured for chlorpyrifos. For cholinesterase inhibition, the halfway point for chlorpyrifos oxon is approximately 1 to 3 nanomolar. This has been published. And my own lab has replicated those results. It is an extremely potent inhibitor the catalytic activity of acetylcholinesterase. Let me give you a little bit of a comparison.

One of the widely used drugs in controlling Alzheimer's symptoms is tacrine. It is not an organophosphate, but its target is acetylcholinesterase in the brain.

It's affinity, tacrine, for acetylcholinesterase is about 100 nanomolars. So this is a prescription drug that has been designed to target brain cholinesterase. And its potency, at the acetylcholinesterase, is about 100

nanomolar. Chlorpyrifos oxon is about 100 times more potent than tacrine.

And that's the other distinguishing feature that once it binds, it doesn't come off. It's irreversible. So whereas, you can expect effects with tacrine to wane, when you stop exposure, the effects of chlorpyrifos oxon will be persistent until the enzyme turns over.

And there are indications that acetylcholine turns over rapidly, and so you can have both spontaneous reactivation, but also replacement of acetylcholinesterase that's bound to the chlorpyrifos oxon. That's phosphorylated by the chemical.

So in terms of the non-catalytic activity though, those seem to occur at much lower levels. They occur somewhere in the picomolar to nanomolar range. So that's about 100- to a 1000-fold shift in potency. Those can be reviewed, if we go through mechanisms. So I'm not going to belabor the point, but the data seems to be quite strong, in terms of the morphogenic effects chlorpyrifos.

Other targets that were discussed in the materials that were handed out are endocannabinoid as a mechanism the fatty acid metabolism that leads to endocannabinoid synthesis. In particular, the enzyme FAAH and MAG lipase. These are enzymes that process endocannabinoids in the central nervous system, as well as

other neurotransmitters such as serotonin and more general mechanisms, which could involve any mechanism actually, such as oxidative stress. But those seem to be less sensitive targets in general to CPF and CPFO modification than either the catalytic functions of acetylcholinesterase or its morphogenic functions.

So now I'm going to get to the behavioral data, and because there were well over 300 papers, I'm actually going to summarize quite a bit.

(Laughter.)

COMMITTEE MEMBER PESSAH: So yes. They are.

So what I'm about to handout, and the table being projected, are from Burke et al. just published a few months ago, which really didn't summarize 300 papers, but they summarized trends in the data from animal studies. They also point out their relationship to the human studies, but I'm only going to focus on the animal studies.

And when you review this, there are two ways that you can look at it. You can look at it that nothing is consistent from study to study. But here are the facts that need to be taken into account, at least the facts that I took into account.

Route of exposure. With animals, you have quite a bit of liberty. You can decide on an oral route, and

that oral route can either be through feeding studies, but more frequently it's a gavage study. And so you're handling the animal and forcing the material orally so that it's bioavailable to the animal, a much more accurate way of doing things, than feeding studies, but, in fact, there's a lot of stress involved with gavage.

I.P. injections have also -- are part of this data set. The most recent is a subcutaneous route which has many benefits, because apparently if you administer chlorpyrifos subcutaneously, its distribution and pharmacokinetics are much more likely to reflect dermal exposure, which is the main route of human exposure, at least in applicators and farm workers, but also, I believe in -- another route is oral exposure through food contamination, but mainly through dermal exposure.

So when we look at the animal studies, what I focused on were animal studies that actually were within 1- to 10-fold of the benchmark response modeling doses that EPA has proposed. And these are based on a 10 percent drop in blood or brain cholinesterase. And so as you can imagine, if you assume that you have a one to three nanomolar affinity for cholinesterase, that, in fact, a 10 percent drop is a quite sensitive endpoint. And the point was made that the BMR is based on 10 percent, because it's a very reliable measure, that you

can actually measure a 10 percent drop in cholinesterase.

And so the BMR -- I'm sorry, the BMD for these types measures, if you read the literature and the material that was set out, ranges between 1.3 and 1.5 mg/kg per day. And so if you want a study that really reflects the BMD, you want to look at studies that are within a 10-fold in rats and mice.

And the reason that you have to go up to 10-fold is that it turns out that rats and mice have much higher protective mechanisms to detoxify or prevent the toxicity of chlorpyrifos than do humans. Okay. And I can -- I can -- we can discuss that a little bit later as well.

So the species used are typically rats and mice. And as I just mentioned, they have a very high level of circulating cholinesterase. These are not acetylcholinesterase, these are what are a called pseudocholinesterase. And they are in the blood, and they act like a sponge to absorb things like organophosphates, but other compounds as well, and reduce the bioavailability of organophosphates such as chlorpyrifos.

And so because of this, and several other factors, a recent set of experiments has been initiated in guinea pigs, which actually have much lower levels of chlorpyrifos, detoxifying mechanism that are much closer to humans. And one of these studies is actually

highlighted in this review, which shows that guinea pigs are, in fact, more sensitive to chlorpyrifos, both in terms of developmental neurotoxicity, but also in imaging studies where brain imaging shows differences in connectivity in the brain subsequent to chlorpyrifos exposure.

So what can be said about the virtually hundreds of peer-reviewed papers that have demonstrated motor and/or cognitive deficit with gestational or early-life exposures to chlorpyrifos.

Well, rats and mice studies within the 10-fold limit of the BMD, which is 1 to 10 mg/kg per day, have consistently showed differences from their respective vehicle control groups in behavioral outcomes. Now, if you go across those studies, they don't all show the same level of responses. And that's probably because that studies don't replicate identically with respect to timing of exposure, when the measurements were made, how the measurements were made.

One can sort of draw an analogy here. If you find a gene in a population that's highly correlated, in fact, geneticists would say are causative for a developmental disorder, and you model that gene in a mouse, would you expect to see the exact phenotype in the mouse that you see in the human population that's

affected?

And the answer is absolutely not, because mice are -- have their own genetic background, and putting a susceptibility gene in a mouse may recapitulate some of the molecular and cellar elements of the disorder that you see in humans, but may not be a phenocopy. And the same can be said about studies with chlorpyrifos that vary in terms of the exposure window, the route of administration, and several other factors that complicate, and what measures were made, and how they were made.

And so -- but, in general, what you can glean from many of these papers is that there is a change in locomotor activity in mice and rats subjected to different developmental paradigms of chlorpyrifos exposure. And they sometimes correlate with cholinesterase and acetylcholinesterase inhibition, and other times they don't. But that's to be expected if there are multiple mechanisms that can occur.

Other studies have also shown developmental exposure to rats and mice in different vehicles and routes of administration, produce spatial learning and memory deficits. And those deficits can, on occasion, be sexually dimorphic. That is that males and females respond differently, which suggests that there are specific challenges to understanding how mechanisms relate

to behavioral outcomes.

Whether the impairments are more pronounced in males and females really depends on the time at which the animals are exposed to CPF. In general, subacute exposures in rats and mice to CPF seem exclusively during the early prenatal period, seemed to produce cognitive deficits that are more pronounced in females than in males.

In contrast, cognitive deficits resulting from neonatal, with or without prenatal exposure in rats to CPF are more pronounced among males than females. So there is this dichotomy, and one needs to wade through the literature.

The bottom line though, as I saw it, in reading all these papers is there's the consistent theme here where prenatal, perinatal, or postnatal exposure to chlorpyrifos in the neighborhood of 1 to 10 mg/kg per day, tends to produce measurable behavioral changes relative to the controls in those studies. Some studies are stronger than others, but many of these studies are actually pretty strong, and are performed by labs that are well versed in animal behavior.

And so if you look at Table 1 here, the locomotor phenotypes range from no response to increase in locomotor activity to decrease in locomotor activity. And these

need to be associated with other -- what other measurements were made with these same cohorts of animals in order to get a full picture. If you were to look just at this table of these studies, one would say well, you know, it's really confusing that there could be increases, there could be no changes, and there could be decreases.

But if you look carefully, the exposure paradigms are not all the same, the species are different, but yet one of the things that you can go across and say most of the studies, within reasonable dose -- dosages, produce changes in motor activity.

Can we go to the next one?

--o0o--

COMMITTEE MEMBER PESSAH: Again, this is just a subset of cognitive phenotypes that have been associated with exposures, again in the BMR range of doses, 1 to 5 -- in this case 1 to 6. But as you read across, mice and rats show changes in spatial learning memory, and these are sex difference -- there are sex differences. And these reflect the wide diversity of the data that actually was reviewed by OEHHA and presented in all the documents that we received.

So I'm going to stop there. I have some more specific examples of recent literature to go through them. But I think I may be good to stop here and take questions

or go on to second.

CHAIRPERSON GOLD: Does the Panel have any questions for Dr. Pessah?

Okay. Then I think we'll go to the second discussant. Dr. Luderer.

COMMITTEE MEMBER LUDERER: Thank you, Dr. Pessah, for that wonderful and very detailed overview.

I think what I'm going to do, since Dr. Pessah gave this wonderful overview, is that I'd like to focus on what I think are some of the key neurobehavioral, as well as some of the structural and neurochemical endpoints as well and just kind of highlight those.

So as Dr. Pessah said, there are several hundred experimental studies that examined the developmental and neurobehavioral toxicity of CPF. And what I'd like to do is talk first about what I see as the strength of the database as a whole, since this is a very large database.

So as you've already heard, the database includes multiple studies each for early and late gestational, as well as early and late postnatal developmental exposure windows. And we know the early postnatal exposure windows are relevant to -- or analogous to in utero exposure in humans.

Many of these studies included doses that minimally suppress brain cholinesterase activities and

some -- activity, and some included doses that do not suppress brain cholinesterase activity, which I think is relevant for talking about some of the other possible mechanisms that Dr. Pessah mentioned.

Even when the -- and it's also important to note that when these cholinesterase inhibiting doses were used, effects were measured long after the exposure, and therefore after the cholinesterase inhibition had ended. And most -- many of the studies measured endpoints not only post-exposure, but also well into adulthood.

Many, if not most, of the studies had additional strengths. They randomized the dams and/or the pups to treatment groups. Endpoints were assessed by investigators blind to experimental groups. Many of the studies of developmental exposure also standardized litter size, and as well as randomly cross-fostering pups shortly after birth to avoid dam effects. And most of the studies measured endpoints in both male and female offspring.

One thing that, as a female reproductive toxicologist, I noticed that it is I think a weakness of the studies is that none of -- almost none of the studies controlled for estrous cycle in the females.

However, this would be expected to increase variability within the female groups, and therefore it would decrease the power to detect treatment related

differences. So I think we're -- the fact that differences or affects were observed in females, they may have been stronger if they had been controlling for estrous cycle stage.

Most of the studies did not mention blinding of personnel to treatment during dosing, but given that the investigators were generally blind to treatment, I don't think this is an important weakness. The most common exposure routes, as already discussed, were oral gavage, generally in an oil vehicle or subcutaneous injections in dimethyl sulfoxide. And we've already heard a little bit about this route, but acknowledging that subcutaneous injection is not an exposure route, relative to humans, but it does mimic -- not relevant to humans directly, but it mimics human dermal exposure.

I think it's also important to highlight a pharmacokinetic study by Marty et al. from 2007 that found very similar pharmacokinetics between subcutaneous injection of CPF and DMSO, and gavage administration of CPF in rat milk. While there was a -- some difference in both the Cmax and the area under the curve, they were both lower and the half-life was a bit long with subcutaneous injection of CPF and DMSO compared to a gavage administration in corn oil, but I think still relatively similar.

So the largest database in terms of studies examining the same endpoints in multiple studies, we've already seen some of those, is for two tests of cognition, the radial arm maze and the Morris water maze. So the radial arm maze has been used to test the effects of CPF exposure on cognition during multiple developmental windows with one -- with multiple studies from one group from Duke University, the Slotkin group.

And they -- I'm just going to summarize some of these. So in Icenogle et al. 2004, they found deficits in male and female rats after early gestational exposure. With late gestational exposure in 2002, they found deficits in female but not male rats. And with early postnatal, postnatal day 1 to 4, deficits in males and improved performance in females.

So recently, two other groups have reported similar -- similarly reported deficits in male rats, and improvements in cognition in females with exposure from postnatal day 1 to 21 - that's Johnson et al. from 2009, and with exposure from gestational day 7 through 21, so a wider window, that's Gomez-Jimenez 2017.

And so this provides independent confirmation of effects of developmental chlorpyrifos in the radial arm maze at least during those developmental windows.

Now, only one group has reported on radial arm

maze testing in mice after late gestational exposure. And they reported no effects in either sex. That was Haviland et al., 2010. But they also observed very low initial error rates in that study, and no decrease in error rates over testing sessions and controls, which you usually see in this test. It may have had something to do with the construction of their maze, which was 8 arms versus 16 arms. Maybe someone that does this test regularly could comment on that. But to me, that decreased my confidence in those results.

So the Morris water maze is another cognitive test that has been used in multiple chlorpyrifos neurodevelopmental studies in three different species, all of which show deficits with different exposure windows. So early gestational exposure caused deficits in male and female mice in studies from two different groups. That was Billauer-Haimanovitch, et al. from 2009, and Turgeman at al. from 2011. Late gestational exposure caused deficits in male and female guinea pigs, two studies from Mamczarz 2016, and Mullins et al. 2015, and late postnatal exposure caused deficits in male and female rats. That was Jett et al., 2010.

Exposure of rats during gestation through lactation, so again a broader developmental window, caused deficits in female but not male offspring in the

Gomez-Jimenez study. And in all of the studies they did not test other developmental windows. Those were the developmental windows that were tested in the studies.

A number of other studies, an endpoint where I think there is also some consistency, as well as some variability, is various tests of anxiety and emotion. So only the elevated plus maze, and the light-dark box tests of anxiety have been used in multiple studies across multiple developmental windows. Female offspring that were exposed gestational day 15 - these were mouse offspring -- 15 through postnatal day 14 to CPF displayed increased anxiety-like behaviors in both tests. In that study males were not tested. That was Braquenier et al. 2010.

Another group found that gestational day 14 to 17 exposure increase anxiety in female, but not male mouse offspring. That was using the light-dark box, Venerosi et al., 2010, while the same group using a different test, the elevated plus maze with exposure to CPF from gestational day 15 to 18 found that exposure decreased anxiety in females with no effect on males. So opposite effect on females, but two different tests in similar exposure windows.

Early gestational exposure from the Slotkin group and Icenogle, et al. had no effect on elevated plus maze

performance in rats.

Moving to the early postnatal exposure, this led to decreased anxiety in female rats, but not males using the elevated plus maze as a test. This is the -- this Aldridge et al., 2005. And late postnatal exposure decreased anxiety in male but not -- in female but not male mice, again by the elevated plus maze, and in both sexes by the light dark box. And this is with the Italian group, the Istituto di Sanità in Rome.

Overall, I think the literature on the tests of anxiety and -- I wanted member one -- mention one other that didn't use the light dark box, but did use a similar test, which assessed the likelihood of rats moving out of a dark safe place into the light. And this is a recent study by Carr et al. from 2017, that included two doses that didn't decrease cholinesterase activity in the brains, and that found significant changes at those doses, so -- in both sexes.

So overall, females were affected more than males with mid-gestation through late postnatal exposures, but the direction of effect was not always consistent between the two tests for the same developmental window.

Finally, I wanted to talk a bit about the -- as far as behavioral testing goes, about social behavior interaction tests. That was largely tested by one group

from Italy, the Istituto Superiore di Sanità that I mentioned. And this body of work shows that mid-gestation through late postnatal exposures in mice increased male:male and female:female social investigation and solicitation behavior, and increased male:male aggressive behavior, while decreasing maternal female aggressive behavior against an unknown male, and increasing investigation behavior of that male.

So in addition to these and other -- we already heard about some of the motor endpoints. In addition to these behavioral endpoints, many studies have examined the effects of developmental chlorpyrifos exposure on the structural and neurochemical development of the brain, and some of those have been mentioned as well.

So this range ranges from persistent morphometric changes, such as decreased size Of the parietal cortex in rats after perinatal exposure to five milligrams per kilogram in Hoberman, decreases in the number of neurons and glia in various subregions of the prefrontal cortex after early gestational exposure to 5 milligrams per kilogram per day in mice. And these brain MRI imaging changes in guinea pigs that were mentioned by Dr. Pessah with decreased forebrain and striatal volume, and decreased amygdala and striatum diffusion parameters.

The Duke group has documented fetal -- that fetal

or neonatal exposure to CPF disrupts neuronal differentiation, and replication causing loss of neurons and deficiencies in synaptic transmission. They've shown persistent effects of developmental CPF exposure on serotonin -- serotonergic, dopaminergic, noradrenergic, and cholinergic systems in the rat brain following gestational and early postnatal exposures to 1 and 5 milligrams per kilogram CPF. So these are numerous studies by Slotkin et al. and Slotkin and Seidler.

Changes include alterations in neurotransmitters, neurotransmitter receptors and transporters, and alterations in turnover rates that correlate with behavioral changes that were observed during the same dosing window.

In addition, some papers by Carr et al. -- several papers show that the developing brain is -- appears to be even more sensitive to disruption of enzymes that are involved in the cannabinoid system, so that these are enzymes that degrade the cannabinoids. And so these -- which are inhibited leading to increased cannabinoid concentrations in the brain. And this occurs at doses of 0.5 and 0.75 milligram per kilogram per day, at which in the same animals no brain cholinesterase inhibition was seen.

So finally, just in conclusion, there is a large

body of literature investigating the neurobehavioral, structural, and neurochemical sequelae of developmental exposure to CPF. And these studies utilize different prenatal and postnatal exposure windows, doses, dosing routes, species and strains, and behavioral endpoints. And some of the key findings that I tried to highlight have been replicated within and among laboratories, and have been documented in more than one species.

So overall, I think that the weight of the evidence supports that CPF is a developmental neurotoxicant, including at doses that do not or minimally only suppress acetylcholinesterase activity in the brain.

CHAIRPERSON GOLD: Thank you, Dr. Luderer. Any questions for this discussant?

Okay. So next we are going to talk about animal studies of other developmental endpoints, and our first discussant is Dr. Plopper.

COMMITTEE MEMBER PLOPPER: This doesn't work.

Is that working?

It's on. There we go.

Okay. As you heard, most of the literature concerning this compound is focused on neurodevelopment. And our charge was to look at the non-neurodevelopmental studies. And what I want to do is discuss two different areas. But first, I wanted to emphasize something that

OEHHA did with their 2008 report, which was excellent, which was a thorough review of what happens to a fetus when it is exposed in the mother, and assessments of how this will impact the success of the fetus in the mother, and then postnatally.

And what I wanted to emphasize, which they've already reviewed, but I think it's worth keeping in mind as we go through these studies is that one of the challenges is what is the impact that the exposure has on the pregnant mother?

And the -- these studies did a very complete job of analyzing what -- or showing what these could possibly be. And obviously, if we focus on maternal toxicity with these exposures, you want to look at -- at death is the first thing, and then loss of body weight or failure to gain body weight. And that was considered to be the first criteria. And if you look at most of these neurotox studies, they say, well, it didn't have a negative effect on the mothers, because they didn't lose the weight.

The other thing that was used, and I want to emphasize it, is that cholinergic overstimulation is also another criteria that's been used to establish whether this is toxic to the mother. And some of the things that were used were things such as shaking, lachrymation, exophthalmos, diarrhea, tremor, those sorts of things. If

that was the case, then those were considered to be maternal toxic, so anything that happened to the fetus would be the result of something that was toxic to the mother not to the fetus.

And then they looked at two separate sets of categories, and I won't go through them all. But it's at reproductive and fetal parameters that includes how many fetuses. You've got to remember a lot of these studies are done, they impregnate the female and they identify that the, whether rats or mice or another species when it's impregnated, then often the exposure starts. So it's also compromising the ability of a fertilized ova to actually inhibit -- inhabit the uterus.

So there's assessment of how many of these implants were actually successful, what was the corpora lutea function. And how many of these fetuses then came out live, what percentage were successful and which were not, as well as was there a difference in the sex ratio.

And then they also went through three or four of these studies. I won't mention them all, but I wanted to emphasize that they use external assessments of the fetus once born or when it's taken out of the pregnant uterus to decide whether there was a fetal toxicity approach, something that was negative, and usually starts with an external examination.

And I didn't read through all 360 of those, but when I was trying to find things that didn't have something to do with neurotoxicity, one of the things that's always an issue well they all looked healthy.

Well, what does this mean?

For one thing it means that they were all about the same body weight, they were all about the same crown-rump length. And when they looked at the -- at the external morphology, they found that all of the appendages were there, including the fing -- the digits, and the tail. And then they would look at the head and see if it was malformed, or if there was some kind of a cleft palate, or whether there was some irregularity with the eyes.

And this is what they used. Now, when I started, I said, well, how -- what does this mean in terms of what's going on with the rest of the fetus? Well, there were three or four studies where they actually did a very detailed assessment of all of the internal tissue organization, just subgross not histologically. And what they established is if the outside appears to be healthy, then there is no disruption of organogenesis.

So this was the types of things -- I guess the only thing that was -- that ever came out in any of these studies, and it was only at doses that the experimenters

considered to be toxic to the mother, based on their toxicity criteria, was a failure for successful ossification of some of the bones, specifically sternebrae.

Other than that, there's a mass of these studies, and most of what you've heard about, these were these unconscious or semi-conscious assessments that do something to the fetus.

So there was a lot of studies there. And I think the bottom line for most of them was that the conclusion was that the fetal exposure on a pregnant mother does not affect the successful non-neurodevelopment of the fetus.

Okay. And that was -- that's -- I can -- if someone else would like to comment on that later, they can. But that seemed to be the basis for most of these. And some of these studies followed these. There were three studies that did multi-generational studies, and maternal exposure allowing these offspring to grow to maturity, and sexually reproduce. And then one study did it twice, so it was an F2 generation, did not affect that part. So the conclusion was that this was probably not a reproductive toxicant, in terms of those things.

Now, in terms of other things, I would like to follow up on Dr. Pessah's. He's already said about three-quarters of what I was going to say about

metabolism, but I would like to emphasize two things that there are two -- that there was two studies that looked at metabolism of something other than the nervous system, and that was the liver.

And this should be a major concern, because it's the liver of these fetuses that deals with most of the xenobiotic compounds that an animal is exposed to. And one study by -- on mice by Buratti and did pre-exposure -- or, I mean, prenatal exposures from gestational day 15 to 18, and then assessed a variety of postnatal time points up to 150 days, and they also did a postnatal exposure.

And I'm not sure if you consider this -- it's 11 to 14 days, so it's kind of on the border, and I won't go through all the details. But what they assessed in the livers was, number one, what's the capability of the liver to actually convert chlorpyrifos to the oxon? And then what is -- how is the aromatases changed, and then assessment of cytochrome P450 expression and function. And there are about 12 of them. I'm not going to go through them all. But these are the key enzyme systems that actually metabolize xenobiotics.

And they used, as their model compound, testosterone hydroxylation. And what they found is that out of these 8 to 10 that they measured, they found that six of them were -- their function was significantly

modified at both of the concentration levels for exposure, which were the -- in the lower end of the range that Dr. Pessah mentioned as being the ones that they would use, 3 and 6 milligrams per kilogram.

And, of course, they assessed everything first, and there was nothing wrong with these babies, except that they couldn't metabolize. And that -- I think that is a major concern. There was changes in the aromatase went down, as well as the ability to convert to the oxon, and then all of these P450s that were changed. And that was a fairly complete study. The only concern I had with it is the animal numbers were not great, but they followed them out to 150 days. And it was only at a hundred -- in the 150-day group that most of these changes had come back to a steady state that didn't make any difference whether they were exposed or not.

So it does suggest that the thing that was of concern is that when they did the postnatal treatments, which might be considered to be too far along the developmental path for humans, then these things didn't reverse well.

So what this -- I interpreted this to mean that this was probably the most thorough metabolic study that I could find that it did not reverse well, and that so continual exposure prenatally versus postnatally may have

a negative impact on the whole cytochrome P450 system, which would be -- would have negative impacts for all sorts of other metabolic processes, including every other toxicant that an individual would be exposed to.

I would point out that there was another study that was mostly a neurotox study, but they also looked at the conversion of the -- to the oxon and for both cholinesterase and the carboxyhydrolase that's by Lassiter. And most of it's tucked in among all the neuro things, but they found essentially the same thing. The treatments were prenatal and the impact on liver function for both of these enzymes was all -- as much as 50 percent inhibition that continued. It didn't change back. So something had changed the metabolic capabilities of the livers.

And the other area that I think is worth considering is the thyroid. And there's not -- the two studies that I'm thinking of did not -- that I evaluated did not have really detailed information, because this was not necessarily a primary subject. But both -- for the study on by Se Angeles in mice showed that there was a change -- a negative change in thyroid production and some indication of histopathology in the thyroids from prenatally exposed animals in the same dose range that is considered to be nontoxic in mice.

And there was a similar study by Haviland, which is primarily a neurobehavioral study, but they did have the last part of it had some information indicating that there wasn't a negative impact on the ability to produce thyroxine T3, T4, and the uptake.

And so with that, I will stop.

CHAIRPERSON GOLD: Thank you.

Any questions for Dr. Plopper?

Seeing none.

Dr. Allard is the second discussant on this topic.

COMMITTEE MEMBER ALLARD: All right. Thank you very much. Can everybody hear me well?

Okay. So right -- so my role as second discussant, is to look at animal studies that pertain to other, i.e. non-neurological developmental endpoints. So I reviewed many studies. Most of them covered in the 2008 OEHHA hazard identification document. And those include studies in many accepted models of toxicity, such as rats, mice, and rabbits.

But because there are also a lot of discussions, as we've already heard this morning, as to whether fetal developmental effect can be observed independently of maternal toxicity, I also considered several studies in zebrafish, where development is external to the mother.

Therefore, recommended this issue of marginal toxicity versus embryonic toxicity.

And I would like also to briefly discuss some publicly available data from the ToxCast program that includes teratological endpoints also generated in zebrafish, which I think will illuminate a little bit the discussions this morning.

So I will give you sort of my broad overview of the findings from reviewing the literature, is that the outcome of chlorpyrifos or chlorpyrifos oxon on teratological endpoints was somewhat heterogeneous. So we'll just pull out what to me was actually a significant example. There are studies where gestational exposures in rats by gavage, so performed through gestation, led to a decrease in size and weight in one study by Condette et al. in 2015, but actually an increase in weight in another study at the same dose done through the following the same protocol. And actually, the authors are shared between the two papers, and that's by Reygner et al. in 2016.

So this is -- to me, was kind of an illustration of the dichotomy and the viability between the studies. There's one very interesting study by Mansour and Gamet-Payrastre in 2014 done in the mouse. Also gestational exposure to very low levels of chlorpyrifos estimated to be 0.01 milligram per kilogram per day.

They saw a non-significant decrease in the weight of the pup -- of the pups at birth. So it was a trend. However, they saw a dramatic and significant reduction in the size of the spleen and enlargement of the liver, which later developed to have abnormal pathology, specifically of the liver.

So I felt this study was compelling, but of course needs repetition. And I also need to point out that there was some mistakes in the annotations of the tables unfortunately, with regards to the level of statistical significance. So I definitely felt that this study needed repetition.

Going towards the zebrafish though, I felt that the results were more consistent, and to be honest concerning. And they were consistent across different studies performed by different labs, in looking at chlorpyrifos and chlorpyrifos oxon in zebrafish. So, in particular, there's a study by Ducharme et al. in 2015, where they -- it's actually a meta study. They looked at many different chemicals and they actually ranked chlorpyrifos number 6 in its teratogenicity, although their endpoint was behavioral. It was not necessarily malformations of the embryo.

However, other studies have looked at non-neurological endpoints. So a study by Jin et al. in

2015 reported a reduction in body length at all concentrations that they tested, as well as at high doses spinal deformities, and pericardial edema. These outcomes were actually similar to what has been reported by other groups such as the ToxCast -- the data I presented in the ToxCast data sets, in particular by the National Health and Environmental Effects Research Laboratory, where they reported activity of chlorpyrifos oxon towards teratogenic endpoints with an AC50 below the micromolar level. So they were at 0.41 micromolar level for the active concentration, AC50.

And they had a lower exposure level towards a variety of teratological endpoints, such as yolk-sac edema, actual defects, circulation defects, truncation of the body that were in the nanomolar range, so 64 nanomolar for the lowest effect level, and mortality is only observed at much higher levels, 10 times that level. So the LEL for mortality is 640 nanomolar.

I also felt that the truncated body endpoint seen in zebrafish was interesting, because it was consistent with reported teratogenic effects in some of the studies. So the Rubin et al. in 1987 reported some -- sorry, reduced length of the animals. There's also some reports of this in the mouse by Deacon et al. in rats, by Condette et al., where there's that body length reduction.

Overall, however, with regards to these non-neurological endpoints, I believe that there's definitely cause for concern, but I also felt that there was no necessarily unanimous and unified picture here that emerged from the various studies that are reviewed.

It's also unclear what the mode of action - and I guess we'll discuss this later on -- or AOP could be for such teratogenic effects, but I don't think that we necessarily need to understand those to trust or doubt the studies that I mentioned.

And I will end my comments here.

CHAIRPERSON GOLD: Thank you very much.

Any questions for Dr. Allard from the Committee?

Okay. Next, we're going to have a summary of the mechanistic studies. And the first discussant is Dr. Auyeng-Kim.

COMMITTEE MEMBER AUYEUNG-KIM: Hello.

Thank you. So I'm going to discuss the -- summarize the mechanistic studies. I pretty much -- I relied on the EPA reports, which summarized the different -- the EPA human health assessment reports, which stated that there's numerous in vivo and in vitro studies that have been conducted on the possible mechanism aspects of the neurodevelopment effects.

And so although that there are several different

mechanisms that have been postulated, that there is no direct -- or no definitive mode of action or adverse outcome pathway that has been identified.

And as Dr. Pessah as well Dr. Luderer mentioned that the plausible hypothesis of some of the mechanisms are that CPF causes alterations in the acetylcholinesterase structure resulting in a acetylcholinesterase acting as a morphogen that influences the growth of cells during neurodevelopment.

CPF can also act directly by singling through the muscarinic or nictonic cholinergic receptors to regulate neural cell proliferation and differentiation.

CPF can also produce reactive oxygen species, resulting in neuronal cell damage caused by oxidative stress. And CPF can cause alterations in serotonergic nervous system, resulting in acute and/or permanent changes to the neuronal cells.

And then -- and a review of -- oh, and then also newer research has postulated that CPF affects the tubule -- microtubule-associated proteins and axonal transport, which are integral to the nervous system development and maintenance.

However, there is no experimental evidence that the perturbations of these endpoints during the development has neurotoxic outcomes. I also reviewed

additional literature that was provided to us since the EPA report. Several of those mechanistic studies were model development. And so they were not necessarily proven in vitro models, one being the avian chick model, and also some stem cell models.

But, in general, most of these studies they -- they did not -- they provided some information as far as what are potential mechanisms, but no direct evidence as far as what could be the mechanistic cause for the neurotoxic -- neurodevelopmental effects. So...

CHAIRPERSON GOLD: Thank you.

Any questions?

Okay. And, Dr. Allard, you're up again as the second discussant on mechanistic.

COMMITTEE MEMBER ALLARD: All right. Thank you very much. So I -- as we've already heard this morning, I think historically the biological activity of chlorpyrifos and its oxon, in particular, have been best understood through the inhibition of acetylcholinesterase.

And what I think is also clear from what we've already heard this morning, and also the various documents that were provided, is that now there's going concern with regards to the ability of chlorpyrifos, and chlorpyrifos oxon to act through other non-acetylcholinesterase mediated mechanisms.

So some of these were just mentioned. I'd like to mention oxidative stress. There's some evidence for epigenetic, specifically DNA methylation mechanisms. Although, to be honest, I didn't find these -- I found that these studies needed repetition.

There's also some also described effects on DNA synthesis. Although, my evaluation of these studies, especially the DNA synthesis. Some of the DNA synthesis studies was that the concentrations used were in vitro were quite high, in the -- in one particular study was up to 30 micromolar in vitro.

So I -- I'd like to go back to the non-acetylcholinesterase mechanisms a little bit later. But I -- as geneticist, I wanted to understand the potential mechanisms from the lens of what we can expect when we have the complete deletion of all acetylcholinesterase.

So if you look at the mouse knockout, interestingly the phenotypic characterization of the mouse was, from my perspective, done at a sort of a macro scale. There was not a lot of pathology -- detailed pathology performed. But I think the mouse mutant still revealed the fact that the homozygous mutant is lethal very early on during life or early on during life, I should say, during the second week.

The pups seem to be born rather normal again by gross evaluation, but they do not grow properly. They tend to not gain weight. The heterozygous mice, however, are normal, meaning that from those studies, an inhibition down to 50 percent is enough to sustain at his gross morphological normal features, and survival.

What was really interesting about these mice however is that if you challenge them with a second organophosphate, or with a specific inhibitor of butyrylcholinesterase, then the mice die absolutely right away within minutes. And so that sort of gives you an idea of a second hit model where if you had both acetylcholinesterase and butyrylcholinesterase, then you may have a very strong cause for concern.

And this -- this is an important, important point to make, because of what I'm going to say next about the -- what I found by mining the ToxCast database. So looking at mechanisms of toxicity, a lot of studies will have, of course, a working hypothesis. I wanted to go with a more hypothesis-free evaluation of this. And so I -- again, I mine the ToxCast database from EPA.

Just as a reminder, this is a publicly available database of about 700 different high throughput assays that cover a wide range of molecular outputs, and that channel themselves in about 300 signaling pathways. So

you cover a really wide spectrum of different types of molecular endpoints.

What was really nice comparing the outcome from the ToxCast database between chlorpyrifos and chlorpyrifos oxon is that you saw what you expect, and what we've already known now for quite awhile is that chlorpyrifos oxon is much more biologically active than chlorpyrifos by itself. So the AC50 of most of the assays for CPF did not fall below 10 micromolar, whereas with CPFO, or chlorpyrifos oxon, many assays, the AC50 of many assays, fell below 10 and there were actually quite a few that fell below 1 micromolar, again, consistent with what we know.

Another validation of -- to me of that kind of date is one of the strongest hits from the assay was actually inhibition of acetylcholinesterase, specifically the human version of acetylcholinesterase, with an AC50 of 0.35 micromolar.

What was really interesting to me, however, is that that was not the strongest hit in the data. The strongest hit was actually butyrylcholinesterase with an AC50, predicted AC50 of 3.4 nanomolars, so a hundred times less than acetylcholinesterase.

So again, this, to me, kind of goes back to that two-hit hypothesis, that the mouse.... has indicated.

There were also some really interesting and -- hits and also cause for concern with a very strong downregulation of major histocompatibility complex genes, such as HALADRA with an AC50 of about 10 nanomolar. And this was definitely a theme from the data. And ability to inhibit a variety of different CYPs. And I think we've already mentioned this this morning.

So many CYPs seem to be a target of CPFO, of the oxon version chlorpyrifos. And the strongest hits of those was CYP2B6 with an AC50 of 0.4 micromolar, but the effect was very, very large. And CYP2B6, in particular, seemed to be important for the metabolizing of various drugs or pureed anti-cancer, antidepressant, tamoxifen as well.

So in the end, however, I think I'm going to go back to the previous conclusion that we don't necessarily have a clear AOP that emerges from all this, but we have very strong biological signatures that have -- have emerged from the molecular data that again, to me, are cause for concern.

CHAIRPERSON GOLD: Thank you, Dr. Allard.

Any questions for him?

COMMITTEE MEMBER PESSAH: I have one.

CHAIRPERSON GOLD: Dr. Pessah.

COMMITTEE MEMBER PESSAH: I just wanted to point

out that the values that you reported for acetylcholinesterase have to be taken in context of how the assay was done. Because of the irreversible nature of the oxon in inhibiting the enzyme, the amount of time that you expose the oxon to the enzyme, the concentration of the enzyme, and in particular, whether you're in pseudo first order is going to make a huge difference.

When the studies have been done to compare directly under pseudo first order, the IC50, the apparent affinity for oxon peri -- CPFO for acetylcholinesterase is more in the neighborhood of soman and VX. Okay. So it's 1 to 3 nanomolar.

And I think the values I have under those conditions for butyrylcholinesterase are in the 1 to 90 nanomolar. There's a huge variation there. So it's just how the assays are done is very important.

Yeah.

COMMITTEE MEMBER ALLARD: Thank you.

CHAIRPERSON GOLD: Any other comments or questions on this topic?

I'm going to ask the court reporter and the interpreters if they need break?

Yes.

How about 10 minutes -- five minutes. Five minutes. Five minutes.

(Off record: 11:35 a.m.)
(Thereupon a recess was taken.)
(On record: 11:44 a.m.)
CHAIRPERSON GOLD: Thank you.
So to the panel, I'm going to say that I've been informed that the interpreters are having a little trouble hearing us, and it's a little muffled. I think it's -- I've been told it's because we're too close to the microphones, so we've been asked to be a little bit farther away from the microphones.
CHAIRPERSON GOLD: Okay. All right. So finally, the final topic of the Panel this morning is the human studies of developmental effects. And the first discussant is Dr. Carmichael.
COMMITTEE MEMBER CARMICHAEL: Well, that helps. I was going to say give me a signal if you can't -- if it's not sounding right, but hopefully -- hopefully it is.
So, yes, I'm going to review the human epidemiologic literature. There's quite a few fewer than 300 studies here. But nevertheless -- nevertheless a good bit to summarize.
So basically, I'm going to provide a brief summary of findings of the highest -- what I consider the highest quality studies. And the main strengths and limitations of the current knowledge base.

So basically, there have been three main perspective -- prospective cohort epidemiologic studies that I'm going to talk about. And the first one is called the Mothers and Newborn study of North Manhattan and South Bronx performed by the Columbia Center for Children's Environmental Health. We'll call that the Columbia study.

Second one is the Mount Sinai Inner City Toxicants, Child Growth, and Development Study. We'll call that the Mount Sinai study. And third, the Center for Health Assessment of Mothers and Children of Salinas Valley, that's in California, the CHAMACOS study, conducted at UC Berkeley. But given that it has an acronym, we'll refer to that as the CHAMACOS study.

So all three of these were prospective, that means selected prospectively over time, cohort studies. They recruited the mothers during pregnancy. Typically, they were less than halfway through pregnancy when they were recruited. And these infants from these mothers have been followed up through about 11 years of age at this point. So really a wealth of data has been collected for these.

Each of these focused on the association of in utero exposure, so maternal exposure, and neurodevelopmental off -- outcomes and offspring. All three of them have been judged by multiple groups to be of

high quality, for example as detailed in the EPA reports that have been mentioned.

The Columbia study is considered the strongest for our purpose today, because it actually assessed cord blood levels of chlorpyrifos. The others assessed -- the other two assessed urinary metabolites of chlorpyrifos, which would include metabolites of other organophosphates within them.

All of them, as I said, ascertain neuro -- a variety of neurodevelopmental outcomes. They used -- I won't get into the details, but they used very commonly used, highly validated assessment tools. They all enrolled women -- included women at least from around the late nineties, around '97 to '99 at the beginning of their studies.

And this was before the voluntary cancellation of residential use of chlorpyrifos, which occurred in -- around 2000/2001. And the exposure levels were shown to have declined dramatically after that initial recruitment period, which was when the women were pregnant.

So the findings from the Columbia study, prenatal exposures, as they were measured, were associated with delays in mental development, attention disorders, motor development, and intelligence as assessed using various tools, and at various time points from infancy to early

childhood.

The odds ratios are the sort of level of association for a lot of these metrics tended to be relatively strong when comparing the sort of the different ends of the distributions of the chlorpyrifos, seeing 2- to 4-fold increased risks for -- at the high versus low levels.

A dose response was observed for some of the outcomes, such as intelligence measures, and at some time points. It's thought that due to a lot of methodologic strengths, which I'll discuss a little bit more in a minute, these results are unlikely to be false positives. If anything, they could be underestimates, because it's expected that the errors would be non-differential.

So the Mount Sinai and CHAMACOS studies also found that mental -- developmental delays in mental development were associated with increasing levels of maternal urinary levels of chlorpyrifos and other organophosphate metabolites, found somewhat stronger associations at older ages with some of these measures, and also found associations with attention disorders.

So basically, despite varia -- some variability in study design with respect to inclusion criteria, and where the populations came from, and so forth, there is definitely some consistency in positive findings across

multiple domains of neurodevelopment.

So some of the strengths, just to reiterate, of these studies are that they were prospective, and that there was -- so these cohorts were followed over a long period of time, but they kept -- they had good retention rates, so they were able to keep people enrolled in the study, which is helpful to rule out bias.

And they had direct measurement of the chlorpyrifos, or its metabolites in serum or urine. And in particular, I'd like to highlight the Columbia study conducted a number of validation studies to support the cord blood levels as good markers of in utero exposure.

For example, they provided evidence that indicates that this one-time measurement correlated well with urinary measurements of metabolite -- the TCPY metabolites in meconium, and then it correlated well with some studies they did of air concentrations in the home, and maternal urinary levels during pregnancy.

All three studies established that chlorpyrifos levels or the other metrics that they measured as exposure were not confounded by levels of other measured chemicals, such as, for example, lead, methyl mercury, or by other factors, such as socioeconomic status, sociodemographics, or various aspects of the home environment.

Some of the limitations, they're important to

highlight, are that even though it was a direct measurement in serum or urine, it was just one time or at most an average of two times that these things were measured, and does not account for postnatal levels. Although, we do expect reduction in exposure over time due to the residential -- cancellation of residential use.

Also, a limitation is that -- the critical window of susceptibility is uncertain, so the duration and timing of exposure that's needed for particular effects that are being studied is really uncertain for these outcomes. This is particularly challenging for these neurodevelopmental outcomes, because the vulnerable period for the developing human brain can span from early pregnancy into adolescence.

So another potential limitation is that interaction with other chemical exposures or, you know, the effects of exposure to mixtures of chemicals was not -- they really weren't able to assess that to a great extent.

So basically, my summary of the weight of the evidence is that there has been a consistency of associations with neurodevelopmental outcomes across a few -- very strong epidemiologic studies that examined varied populations, used somewhat varied designs, and outcomes and crossed multiple neurodevelopmental domains,

such as cognition, motor control, and social behavioral development.

In addition, this is -- shows some -- bears some consistency with animal studies which have been reviewed by the Panel today. And it has shown that relatively wrong -- relatively strong strength of some of the associations especially in the Columbia study, which with 2- to 4-fold increases in the varied outcome measures, the temporality is clear that the -- that is the outcomes were measured after the exposure occurred.

There's some evidence, as I said, for dose response, especially, for example, for cognition measures in the Columbia study.

A couple -- and just to recap the limitations. Were uncertain about the window of susceptibility, in part because of the long-term development of the brain. Only one of the studies, the Columbia study, which I've highlighted was really -- was able to measure chlorpyrifos directly, and only one time.

And the mechanism of action is uncertain, but certainly many plausible possibilities exist as reviewed also by the panel. So, in conclusion, I'd say there's one particularly strong epidemiologic study, the Columbia study, with support from at least two other very strong studies that I've summarized. There's good biologic

plausibility and experimental support for an association with neurodevelopmental outcomes.

CHAIRPERSON GOLD: Thank you.

Any questions by the Panel for Dr. Carmichael?

So our secondary discussant is Dr. Nazmi.

COMMITTEE MEMBER NAZMI: Thank you.

I don't want to spend too much time reiterating what Dr. Carmichael has already covered, but I want to start with some study design considerations, as we kind of transition from the animal studies to the human studies. And I have three points in terms of study design. One of them is that most of this human data comes from really well designed prospective birth cohorts like Dr. Carmichael was mentioning. And this is, of course, one of the strongest epidemiological approaches that we can take. And I reiterate this, because a lot of these developmental outcomes are really contingent on prenatal and in utero exposures. And it's really important for the kind of time-order relationship of exposure outcome that we know the history and the kind of longitudinal aspect of these -- of the way these data are collected. That's number one.

Number two, I think we should consider the heterogeneity in study findings. And I think this is relevant to the human and the animal studies. This is, of

course, to be expected given the diversity of the participants, their environments, exposure sources, concentrations exposure periods even. But there is a great deal of consistency, like we've heard here in this Panel, across of so many of the different populations even internationally, and in different rural, urban, agricultural settings, which to me speaks to the -- it kind of helps us contextualize the overall effects.

We've seen in the human studies physical, mental, social outcomes examined. And there does seem to be a fair amount of consistency across studies even given some of the heterogeneity in individual population findings.

And my third point about study considerations, I think Dr. Carmichael has covered it pretty sufficiently, so I won't belabor the point, but these -- there are a compelling number of criteria for causation as we talk about in epidemiology as set forth by A.B. Hill that kind of should be mentioned, perhaps most importantly temporality in this concept of birth cord studies; consistency across findings; the strength of the findings, which are pretty compelling in most cases and seem to be consistent across different study populations.

As some of our colleagues mentioned with the mechanistic studies and the animal studies, we have -- we have specificity. In other words, we know that certain

precise exposures have the -- by themselves can lend themselves to developmental consequences and plausibility.

Dr. Carmichael also mentioned experimental evidence. So I think those are -- those are really important to keep in mind. And what I wanted to do was talk about four of the studies -- four of the most recent studies that I thought were -- that I thought were noteworthy, ranging from 2013 to 2017.

And I'm going to begin with the Fortenberry 2013 study. And this is data from -- this is data from Mexican participants, in the Mexican context, and that looked at outcome of ADHD. And there was suggestive evidence for increased ADHD index in the highest -- in the highest tercile among boys. And in some of these studies that I reviewed I think it was clear that there might be some dichotomy in sex.

So it's kind of -- it was kind of interesting that in a lot of these studies there was -- there were some differences by sex. And that study I thought was a good example of that.

The Rauh study from 2000 -- Rauh, I think I'm pronouncing that correctly from 2015, which was among New York participants looked at mid-childhood tremors. And this was notable to me because of the age of the children, which is -- which was approximately 11 years, which

suggested that some of these effects can really have life-long effects, and the outcome was mid-childhood tremors and nervous system consequences as a result of exposure as measured by umbilical cord blood among about 260 minority children.

The next study was the Fluegge study from 2016. And this was among participants in Ohio that looked at mental functioning. And I'm -- I'm giving you a little bit of a diversity of literature since we can't talk about all the studies just to kind of emphasize the different -- the different measures that were -- that were studied.

And finally, the Silver study. This is data from Chinese participants that looked at motor function, reflexes, and locomotion and so on. And in this study, girls appeared to be more sensitive to the negative effects than boys, so -- and I think one thing that I -- that I might like to add in terms of limitations that Dr. Michael -- Carmichael spoke about was was the specificity of -- specificity of the impact on outcomes as relates to things like differences between the sexes.

Besides that, I don't think I have anything further.

CHAIRPERSON GOLD: Thank you. Any questions or comments for Dr. Nazmi?

Dr. Pessah.

COMMITTEE MEMBER PESSAH: So I'm trying to relate the animal studies to the levels of exposure in the Columbia study, which I agree was very well done. And I went to the Rauh 2012 PNAS articles, where the exposures -- the upper quartile -- tertile was compared to the low exposure group, same cohort using FMRI -- or MRI - probably not functional MRI - to measure differences in brain volume, which is a really specific kind of endpoint.

And they found some really amazing differences, which suggest that maybe there is a cause and effect from the cord blood levels to many, many years later. So I went into the cord blood levels, and I did a back-of-the-envelope calculation. And the levels that were included in that study in the high group in the upper tertile ranged in the neighborhood of 4.4 picograms per gram of plasma, cord blood plasma.

So I assume the density of 0.016 and corrected that - it's a minor correction - and came up with the, what we would consider, the PK/PD steady study state level of 13 picomolar.

Now, the question I have is that level is probably not reflective of the peak levels. And that was, I think, a major point that EPA wanted advice on, because they did very, very elegant PK/PD modeling in 2016, which was presented to the FIFRA panel.

So if we assume maybe the peak levels were 100-fold higher than that baseline level of 13 picomolar, that's a pretty astounding dose response, isn't it, that you have that low a level that might peak around 130 picomolar to 1.3 nanomolar to produce these biological effects, again much lower than anything the animal studies.

So then I -- I said, well, you know, let's look at Silver et al. 2017, not as well characterized a study, and I just looked at what their levels of exposure were. And in those kids, because they had cord blood levels, it was 127 times higher for the upper tertile versus the Rauh et al., so in other words, their level of exposures were much higher.

And they definitely saw impairments in the children, but one would imagine if you're 100 plus above in concentration-effect relationship, you would see much more dramatic biological outcomes. And maybe that's the wrong way of thinking about this, but I just was wondering about the very low levels. If, in fact, we believe those levels, which there's no reason to doubt them, that's an amazing potency for producing neurobehavioral effects in the offspring, based on cord blood levels, yeah.

I was just wondering if you could comment.

CHAIRPERSON GOLD: Either of you, Dr. Carmichael,

Dr. Nazmi.

COMMITTEE MEMBER CARMICHAEL: Well, I totally admit, yeah, doing the back-of-the-envelope calculations and so forth is not -- that it's your expertise and not mine. I guess the only thing I would have to add is that I mean there was a -- even though these are -- these are very low levels being measured, there was a lot of variability within the subjects in the levels that were measured. And so they were able to measure -- you know, compare them at different levels, you know, as for the exact translatability of that absolute value, that's where I'm not such an expert.

CHAIRPERSON GOLD: Dr. Nazmi.

COMMITTEE MEMBER NAZMI: Yeah, I think that speaks to two issues, one of them being threshold. And if indeed it's really that low, you know as compared to the animal studies, I think, perhaps it's an alarming finding, and the dose response effect of it. Although, the -- you know, the 127 times higher in one cohort versus the next, it is -- I think it warrants a little -- perhaps a little bit closer examination, but it does speak to perhaps pretty strong -- pretty strong dose response effects. And I think in many of these cohorts, whether they looked at quartiles or terciles at the top, compared to the bottom quartile or tercile was a lot higher. So their exposure,

whether it was source, whether it was frequency concentration, it was -- I think it was -- yeah, sorry. I lost my train of thought.

But I would say dose response and threshold, it speaks to both of those. And if you're -- according to your calculations, I think compared to the animal studies, it is -- that calculation is something I hadn't considered. But if it holds, it's I'd say alarming.

CHAIRPERSON GOLD: Dr. Luderer.

COMMITTEE MEMBER LUDERER: Yeah. I just -- kind of what both of you were saying, I had a couple of thoughts about that. One of them is that this does really, I think, raise the issue as far as mechanism goes, that some of the non-cholinesterase inhibiting mechanisms may be the ones that are at play here, and -- because, as you pointed out, I mean, those levels are not likely to dramatically inhibit cholinesterase activity. And so I think that that -- that's a really -- one possibility.

Another one is that in the human studies, we're talking about hundreds of participants. And, you know, we have less than 10 in most groups in the animal studies. So if we only had 8 humans per group, I don't think we'd be able to see differences in these studies. So, I mean, think that's another thing to keep mind. These were relative large studies that had hundreds of participants.

CHAIRPERSON GOLD: Let me ask the question, would we expect in the non-cholinesterase possibilities that there would be species differences, such that the humans might be more sensitive? Does anyone know?

Okay, then.

So if I can take a moment, I think we're ready to move to public comments. I will say we had on this topic three requests for extra time, which we granted. One of those has had to leave though, and that's Dr. Irva Hertz-Picciotto. And she has left her slides, and we will enter them into the record and make copies of them for distribution.

But if you'll just give me a minute to organize the other ones, then we'll begin with the other public comments.

(Pause in the proceedings.)

CHAIRPERSON GOLD: So I think what we'll do is begin with those who have extended time. But I -- one of those also has to leave early, so if we can switch the order.

MR. LANDFAIR: Chairman Gold.

CHAIRPERSON GOLD: You want to put your microphone on, please.

You want to identify yourself, please.

MR. LANDFAIR: I'm Stanley Landfair. I'm

representing Dow. I didn't get up to speak. Just ask as a procedural point, if the other speaker left her slides behind, if that's something to be considered by the Panel, may we get a copy so that we can see them?

CHAIRPERSON GOLD: Yes. I think I said that our plan is to make copies and distribute them --

MR. LANDFAIR: Thank you. I missed that

CHAIRPERSON GOLD: -- and enter them into the record.

MR. LANDFAIR: Thanks.

CHAIRPERSON GOLD: Okay. Thank you for the opportunity to clarify. I will note that we have a timer up here that I understand can be seen at the podium. So for the timed comments, it will be the amount of time that they requested and that we granted. And for the five minutes, it will also -- it will be five minutes.

But because we have one person who has to leave, so the first person who requested an extension of time was Miriam Rotkin-Ellman from the NRDC.

MS. ROTKIN-ELLMAN: I'm flexible the whole day.

CHAIRPERSON GOLD: Okay. So if the person who has a time commitment is Kim Harley, is she here?

Okay. You didn't request extra time, you just need -- need to do -- yeah. So why don't you come up first, and then we'll do the other two that have

extensions.

Please introduce yourself and where you're from and.

DR. HARLEY: Is this on?

Okay. Thank you. My name is Kim Harley. I'm a faculty member at the School of Public Health at UC Berkeley, and I'm also one of the lead researchers on the CHAMACOS cohort study that was mentioned just a few minutes ago by Dr. Carmichael.

So that is a cohort study that's examining pesticide exposures and children's development in the human population, an epidemiologic study.

I am an epidemiologist specializing in the reproductive and departmental effects of environmental chemicals. And I've spent the last 18 years of my career investigating the effects of organophosphate pesticides, of which chlorpyrifos is one, on the health of pregnant women and children. And I know that several of our CHAMACOS study papers have already been considered by the Committee.

I'm here today, because I wanted to express my concerns about chlorpyrifos, but also about the whole class of organophosphate pesticides. Our research group has published multiple peer-reviewed papers that suggest that organophosphate pesticides act as developmental

neurotoxicants in humans.

And I wanted to just very briefly summarize some of the findings of our CHAMACOS research. Dr. Carmichael has already alluded to them, but I just wanted to briefly talk about what we found.

The CHAMACOS study is a longitudinal birth cohort study that was started specifically to investigate the effects of organophosphate pesticides of, sorry, chronic low dose exposure to organophosphate pesticides on children's health and neurodevelopment.

In 1999 and 2000, we enrolled 600 pregnant women living in a farmworker community in the Salinas Valley of California. And we've followed these women now for 16 years, the children are more than 16 years old now. We see their children every 1 to 2 years, and we conduct detailed physical exams and neurodevelopmental test batteries with the children.

When the mothers were pregnant, we took urine samples to measure levels of dialkyl phosphate metabolites, which are metabolites of organophosphate pesticides. And I believe Dr. Pessah referred to this earlier. These are not metabolites specific to chlorpyrifos. They're metabolites of the entire class of organophosphate or many of the pesticides within the organophosphate class.

So we are not able to look, in this case, specifically at chlorpyrifos, but we are able to look at the class of chemicals that act in the same mechanism, of which chlorpyrifos is one.

We have found in our study that higher concentrations of these dialkyl phosphate metabolites in urine during pregnancy was associated with mothers having Children who had more abnormal reflexes at birth, poorer mental development index scores at age two, symptoms of pervasive developmental disorder at age two, attention problems, an ADHD behaviors at age five, and lower IQ at age seven.

We've also found some evidence that some individuals maybe more susceptible to these neurodevelopmental effects because of either their PON1 genotype or their PON1 enzyme activity.

We also measured these dialkyl phosphate metabolites in the children as they aged, but we haven't found many associations with childhood exposure. So I think it's important to note that our results suggest that it's the maternal exposure during pregnancy to these organophosphate pesticides that may be impacting neurodevelopment, rather than later childhood exposure. And this speaks to Dr. Carmichael's comment about windows of susceptibility.

One of the challenges in doing epidemiologic research on organophosphate pesticides like chlorpyrifos is measuring exposure during pregnancy. And we didn't want to rely solely on urinary metabolites as our exposure measure, so we've also used California's pesticide use reporting data to calculate how many pounds of chlorpyrifos and other organophosphates were used around the CHAMACOS mothers' homes when they were pregnant.

I don't know that this -- these papers were considered by the Committee, but we have now published papers showing that higher use of organophosphates in general within 1 kilometer of the home during pregnancy is associated with lower IQ at age seven. And we've specifically shown that higher use of chlorpyrifos around the home is associated with poorer verbal skills at age seven.

So, in summary, in the CHAMACOS cohort we fund associations of markers of prenatal exposure to organophosphate pesticides with poorer neurodevelopment in children looking at exposure in two completely different ways with mother's urinary metabolites and with usage within 1 kilometer of the home. And then there are several other -- as we've heard, several other epidemiologic studies by other researchers that really show quite consistent findings with ours.

But I particularly wanted to allude to the studies by Rauh et al. in the Columbia cohort that we've already heard about, because those measure chlorpyrifos exposure in another way, in a third way, which was looking at levels of chlorpyrifos in cord blood. And the Columbia study's findings are actually very similar to ours, even though they used a different way of assessing exposure.

They also found lower IQ in children. They also found attention problems, ADHD, and pervasive developmental disorder symptoms. And they also found morphological changes in the brain on MRI scans which is not something we've done yet, but something that we're looking towards doing in the future.

So at this point, there's quite a robust body of epidemiologic literature showing associations of prenatal exposure to organophosphate pesticides in general and chlorpyrifos in particular with poorer neurodevelopment and behavior in children.

These studies take place in different populations and measure exposure in a variety of different ways. Finally, I just wanted to end by commending the Committee for examining the evidence related to neurodevelopmental and reproductive toxicity of chlorpyrifos, but I want to point out that there are several other organophosphate pesticides that use the same mechanism of action as

chlorpyrifos, chemicals like acephate, malathion --

CHAIRPERSON GOLD: I believe your time is up. Excuse me.

DR. HARLEY: Okay. Can I finish my sentence?

CHAIRPERSON GOLD: Finish your sentence.

DR. HARLEY: Okay. So I just believe that the DART Committee should also be examining these other OP pesticides as well.

CHAIRPERSON GOLD: Okay. Thank you.

Are there any questions from the Panel for Dr. Harley?

Thank you.

I think we will move next to Miriam Rotkin-Ellman, who requested extra time, and so she has 10 minutes.

Please introduce yourself and where you're from.

MS. ROTKIN-ELLMAN: I'm so short. Okay. So I'm Miriam Rotkin-Ellman. I'm a scientist with the Natural Resources Defense Counsel.

(Thereupon an overhead presentation was presented as follows.)

MS. ROTKIN-ELLMAN: I also had slides that I think are coming, if not -- so, well, you don't need a slide to know who I am. So this presentation was done in conjunction with my colleague Jennifer Sass. And NRDC has

been involved and worried about the neurodevelopmental effects of chlorpyrifos for a long time. We work in collaboration with other groups, both at the national level, and at the State level, to advance public health protections for children, particularly during critical windows of development.

--o0o--

MS. ROTKIN-ELLMAN: What button am I pushing here?

Ah. Why doesn't it stay?

Can you do it?

It's pretty quick. Okay. Thank you.

And again, thank you for giving some extra time today. There's no financial interests in the topic of these compounds -- comments.

Next slide.

--o0o--

MS. ROTKIN-ELLMAN: So you all have really dove deep into this extensive body of literature. So my comments are largely going to put some of the conversations that have already taken place into a little bit of a larger context in the broader conversation around the developmental toxicity of chlorpyrifos, not only here in California, but at the national level. You all have interfered -- interacted with a number of these documents,

but we're going to just take one step out and talk about this a little bit in a broader context with some broader themes.

Next slide.

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MS. ROTKIN-ELLMAN: So I think others will probably speak to this largely in the public comment. I'm going to -- the question you have in front of you is of grave public health significance for the State of California. We know we have widespread exposure here in California. And a number of the exposure studies that have been done in California, including biomonitoring, has actually shown elevated levels in pregnant women in California as compared to the national average.

So the question of what is the most sensitive endpoint for this particular pesticide is of critical importance in the State of California for devising public health strategies that provide maximum levels of protection. So once again, I commend you for tackling this body of literature.

Next slide, please.

--o0o--

MS. ROTKIN-ELLMAN: You all have already gone through this. This is the Prop 65 criteria. The component I wanted to note here is that while it's useful

to think about each of these different streams of evidence, whether you're talking about evidence in human epidemiologic studies, or animal studies, or the mechanistic studies, that the whole picture is also incredibly informative.

And rather than comparing the strengths or merits of each of these individuals bodies, I encourage you, and some of these -- some of the presenters have already started this narrative, and I really encourage, when you get to your deliberations, that they not be positioned against each other, but that the commonality between the animal studies and the mechanistic studies and the human studies really be explored in terms of weight of evidence for developmental toxicity.

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MS. ROTKIN-ELLMAN: So since the DART Committee last reviewed the developmental toxicity of chlorpyrifos, this question has been in front of a number of independent scientific reviews. Even the first Science Advisory Panel in 2008 was subsequent to the last DART meeting, and then there were two others subsequent to that.

Each of these Science Advisory Panels engaged with the topic of neurodevelopmental toxicity associated with chlorpyrifos, with a number of different questions, a

number of different angles. But similar to the way your own evaluation of these data, these similarly see a very consistent pattern, noted because it's patterned between the animal studies and the human studies, between each of these panels, so -- on terms of a consistent pattern of developmental toxicity.

I want to talk just a tad about the 2016 Science Advisory Panel, because some of the conclusions of that panel have been a little bit confusing and sometimes misrepresented. And while it was not a consensus panel, and, in fact, was actually engaged very much in much more of a question of the dose response related to the Columbia study than it was the developmental toxicity, they did note a couple of things that are, I think, of importance to this Panel.

One being that the Panel, as a whole, agreed that cholinesterase inhibition was not the most sensitive endpoint, and that what we're looking -- when you're looking for the most sensitive endpoint, it is likely below. And that also a number of the panel -- of the panel members really pointed to, what a number of folks have already mentioned today, was the strength of the Columbia study.

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MS. ROTKIN-ELLMAN: In addition, U.S. EPA has spent the last decade or so spending a lot of energy reviewing organophosphates in general, chlorpyrifos in particular in looking at the data. And you all have seen these documents highlighting a few of the conclusions.

Important to note from the literature review of the -- all the organophosphate pesticides is that conversation between the class that we just heard about from Dr. Harley and the specifics of chlorpyrifos. And that those are both informative towards understanding the developmental toxicity of chlorpyrifos.

Again, we saw in both the 2014 and 2016 human health risk assessments from EPA the importance of addressing neurodevelopment, in particular with the 2016 assessment, that used neurodevelopmental endpoint as the most sensitive endpoint

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MS. ROTKIN-ELLMAN: So outside of the government context, the question of neurodevelopment has concerned a number of other outside experts. American Academy of Pediatrics released this statement in the past year, again drawing on both multiple streams of evidence, epidemiological and toxicological studies, and the strength of the evidence for harm for children.

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MS. ROTKIN-ELLMAN: And in the academic Literature, scientific reviews we've already heard, and you all have seen the Burke et al. paper, again trying -- you know, again integrating multiple streams of evidence, at the same time as reinforcing the power and strength of the human studies, at the same time as seeing it corroborated in animal and mechanistic studies.

For the two OP systematic reviews, although both of them looked at both prenatal and postnatal exposures, both highlighted the strength of the evidence for the prenatal exposure pathway, and particularly in the longitudinal studies, finding consistent patterns of outcomes in children.

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MS. ROTKIN-ELLMAN: I want to just talk briefly about the registrant animal studies. And just as I used this mechanism to show that we're really talking about -- the registrant animal studies are really quite a bit older than the body of literature that we're mostly examining today, and that they've also been critiqued by the Environmental Protection Agency, which is in charge of reviewing those studies.

So I note four animal studies that have been highlighted in Dow's comments were all conducted prior to EPA developing a developmental neurotoxicity protocol, and so were not really designed to identify those effects.

The one that was conducted later than that was classified by EPA as unacceptable, because of not properly -- one of the reasons being not properly quantifying the low-dose effects.

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MS. ROTKIN-ELLMAN: And so lastly on this, and you all have already spoken to this a number of times, I just want to come back to it, that when we're thinking biological plausibility, we're thinking about how to connect the dots between these different streams of evidence. Not having a specific mode of action is not necessarily an impediment for listing under Prop 65.

U.S. EPA, also has a staff, you know, weighed in on this conversation to say that not the lack of establishment of action or adverse outcome pathway doesn't undermine or reduce the confidence in the findings of the epidemiologic studies. We know from looking at other developmental neurotoxicants, such as lead, that we need to take public health action, even when we may not have fully identified the exact mode of action or multiple mode

of actions or efforts outcome pathways.

And then again, these multiple streams of evidence, the mechanisms that some of you have already mentioned that have been summarized in a couple of these pieces do support the biologic plausibility of the outcomes that have been measured in both -- in the human studies.

And then lastly, you know, a colleague likes to say we know that chlorpyrifos is neurotoxic. It's designed to be neurotoxic. We know the developing brain is a time of extreme growth in the development of the child's brain. And the idea that you find a lot of reassurance in the biologic plausibility that an act -- you know, a known toxicant to the neurologic system may have increased potency during development.

And thank you for -- for all your attention towards this literature and giving me some extra time to speak today.

CHAIRPERSON GOLD: Thank you.

Are there any questions from the Panel for this speaker?

Thank you.

So now I need input from the Panel. We can either take a lunch break or we can hear from Dow AgroSciences, but they've requested extra time. They have

three speakers, and we've given them 25 minutes.

So lunch break?

Preferences?

Everybody good for another 25 minutes?

So if the speakers from Dow AgroSciences will come forward, and they have 25 minutes total.

(Thereupon an overhead presentation was Presented as follows.)

MR. LANDFAIR: Thank you, Panel members. Thank you, Dr. Gold. Thank you, Dr. Zeise.

My name is Stan Landfair. I'm from the Dentons law firm. I'm proud to represent Dow AgroSciences. We have two other speakers, Carol Burns, who is an epidemiologist, and Daland Juberg, who is a toxicologist that will speak within their own disciplines. And for the interests of time, they will self-introduce.

We want to start though by thanking for the obvious effort that you have put into this. It's not always the case that we appear before a Panel where it's obvious that so much homework has been done.

Nevertheless, we think the real work is just begin. And as the attorney here, I'd make a disclaimer, I am not going to debate with you about scientific points, but I do want to start with a reminder of why -- that we have a process here.

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MR. LANDFAIR: The criteria for listing, there's a reason why we discuss these. It's because we want to make sure the decision gets made within the rails. And the real test here is written right into the statute, is whether or not the data clearly show that the chlorpyrifos causes reproductive toxicity. And if not, it's your duty not to list it.

It's not a duty to fill in gaps and to speculate regarding a method of action - forgive me - but to -- or to try to hypothesize regarding other questions that the data don't answer. The question for us here is whether the data clearly show that this chlorpyrifos causes developmental toxicity.

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MR. LANDFAIR: So I think I made the point, and I don't need to belabor it, but we go by the weight of the evidence. And there's certain things that will not satisfy the clearly-shown standard. And if we find ourselves saying words like, "Well, the data suggest", "It's likely", or seek, you know, for whatever noble purpose or instinct to apply the precautionary principle, that is an instance that belongs in another proceeding.

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MR. LANDFAIR: The question before us again is

whether or not the data clearly show that the chemical causes reproductive toxicity.

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MR. LANDFAIR: The other point I'd like to make is one of context and perspective, and as Dr. Donald made, context is important here. The principal documents that you've received in the hazard identification materials, the HIM, are -- appear on this registration timeline. As we know this is a registered pesticide. It's subject to ongoing continual reviews. The last review began in 2009. It's ongoing now.

And two of the principal documents that you received in the HIM are the 2014 and 2016 EPA revised human health risk assessments. The story does not end there. And even in the context of this regulatory review, there have been other analyses issued and other documents issued that we want you to have for the benefit to help you round out your thinking, and to evaluate -- you shouldn't be making this in a vacuum. There are other agencies in addition to you - and we consider you an agency evaluating these data. And with all respect, they have reached different conclusions. So different, that we want to make sure you have those documents. We've talked about this with OEHHA.

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MR. LANDFAIR: When this presentation is over, I will give you a letter with these documents as attachments. And just as high spot, this is what they say. The USDA, another respected agency, said they have great concerns about the EPA process, even though the use of the Columbia Center for the -- the Columbia study was criticized by the FIFRA SAP, they went ahead and used it without question.

The latest risk assessment, in EPA's word, fails to show either a causal or dose response relationship. And those are gaps in the data that can't be filled by our informed, and well-informed -- we respect your knowledge. But if the data don't show it, we can't get there by filling gaps.

The 2017, I want to bring you up to date with what EPA says today on the status of its evaluation of chlorpyrifos. It says the science addressing neurodevelopmental effects remain unresolved. And in the context of addressing some other petitions, the EPA has largely walked back from its previous conclusions, and has said this needs further review.

With that, I'll introduce Dr. Burns.

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DR. BURNS: Thank you. My name is Carol Burns. I've been studying chlorpyrifos epidemiology for more than

two decades in my role as an epidemiologist for the Dow Chemical Company. And I've now retired and serving as a consultant.

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DR. BURNS: In 2008, the Panel concluded that the evidence was not sufficient to establish chlorpyrifos as a developmental toxicant. And I would like to put forth the position today that the epidemiology results are, in fact, inconsistent and do not clearly show an effect.

So in the next 10 minutes, I'd like to quickly review interpretation of the new epidemiology publications since 2008. And keeping in mind the Hill guidelines and the importance of the scientific tenet for the reproducibility of findings, so talking about consistency across these publications. But I'd also like to talk briefly about a new interpretation of research.

We hear about the Hill criteria a lot, but increasingly individuals concerned about causal inference are also talking about principles of bias confounding transparency, and most important for public health decision making is quality. So I'd like to talk about that briefly today.

So first of all, what is new interpretation? What does that mean?

And this gets to the concept of exposure

assessment, which is so difficult to do in epidemiology. And there are a couple of approaches that I would like to discuss further today. One is proximity to an application, relying on residence, and how important it is to incorporate validation into these studies.

Secondly, as we heard a lot about biomonitoring, because chlorpyrifos is short-lived in the body, about 24 hours, best practices are recommending to use multiple samples and to estimate error in these assessments. And lastly, the importance of specificity. This is highly relevant for chlorpyrifos in using the metabolites. In looking at consistency across studies, are you looking at organophosphates, or are you looking at the data specific to chlorpyrifos?

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DR. BURNS: So one of the studies that hasn't been discussed yet today, but is in the package is the case control study of autism. And I know the panelists are aware of this method, and it's a great idea to replace the use of questionnaires that's -- that has its own biases, to rely on geocoding of residents and matching that with a known documented information about pesticide use.

However, there's also some limitations to this approach as well. And efforts to validate this has been

used with collecting house dust. And some efforts have been underway to suggest that there are improvements that could be made to this algorithm, such as by incorporating information about weather, behaviors of the homeowners, and characteristics of the home itself, such as having an air conditioner.

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DR. BURNS: But house dust alone isn't enough. It doesn't really tell us about internal dose. Other efforts to evaluate proximity to an application have been done using biomonitoring. And this example of a most recent study -- I don't have a clicker -- demonstrates the study from the Institute of Medicine, in which they collected urine in women and children living within 100 meters of an application, looking off season, and second one during the season, and then immediately after the spray.

And what I'd like you to notice is that the levels of urine concentrations of the metabolite of trichloropyridinol are essentially the same across the different categories.

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DR. BURNS: While biomonitoring is important as a tool for validation, we've also seen it used extensively as a point estimate for exposure.

Here's an example from the Fortenberry study in Mexico city, in which they collected three samples in the first, second, and third trimester. And you can see the women are connected by their own colored dot lines, so you can see the variability across them.

And what's important here to recognize is the reliance on a single sample, such as at or near delivery of the child may not predict at all the levels previously. And this is important to keep in mind that we must first do a good job of evaluating exposure before we start looking at the exposure outcome associations.

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DR. BURNS: So what does this mean with respect to interpretation?

Well, importantly, this puts the CHAMACOS study in a different light. Because they collected two urine samples during pregnancy as well as a blood sample at or near delivery, this is an important component. Furthermore, they had a good attention to collecting field blanks and spikes to determine contamination and stability of the analyte.

In contrast, while we've heard a lot about the strengths of the Columbia study, we must keep in mind that they only collected a single blood sample. Furthermore, knowing that the chlorpyrifos is lipophilic, and I know

from experience -- actually, three experiences, that a woman's body weight changes significantly during pregnancy, that it's really important to control for lipids in this type of study.

Furthermore, the Mount Sinai study also relied on a single urine sample.

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DR. BURNS: Talking quickly about the new studies, our detailed comments are in the written comments. But the new studies looked at infant health, the Bayley Scales looking at both physical and mental development, and other outcomes from age three days up to 14 years. But again, looking at the data across these studies are not consistent.

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DR. BURNS: And I'll show you an example of what I mean by not consistent. So we've heard a lot about the IQ testing. So looking first at the studies that collected more than one sample, which is the CHAMACOS study and the HOME study from Ohio, neither of these studies found a statistically significant association with IQ or working memory. And I'll highlight what DEP is the, diethylphosphate, which is a metabolite of 10 organophosphates, to be differentiated from the diethyl phosphate for all the organophosphates. Notably, the

CHAMACOS study has data on the urine metabolite TCPy but did not report it for this study.

There are three other studies, the Columbia study is one, collected a single sample. So collectively across all five studies, the Columbia study is the only study to show an association.

So to get to the point of dose estimate of the Columbia study, is this study true and valid, or is it a false positive?

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DR. BURNS: So in conclusion, the epidemiology data do not meet the criteria for sufficient evidence in humans. Looking at individual studies, the weak exposure study really limits our interpretation of them individually.

Looking at the group -- the studies as a group, collectively looking at similar adverse associations are not consistent. It's interesting having different interpretations. My interpretation of the CHAMACOS study, it is a stronger study, because of its collecting two biological samples. But looking at their publication specific to chlorpyrifos of their data on urinary TCPy and diethyl phosphate, do not show relationships with exposure and adverse exposure -- adverse effects. Excuse me.

So at this point, I'll turn the presentation over

to Dr. Juberg.

DR. JUBERG: Thank you very much. And I appreciate the opportunity to speak to you today.

My name is Daland Juberg. I'm the chief toxicologist for chlorpyrifos, and have been for the past 12 years.

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DR. JUBERG: Similar to Dr. Burns, in this -- this Panel reviewed the evidence in 2008, and you can see the conclusion there when it was reviewed for both a developmental or reproductive toxicant. It would be my contention that the same evidence has not changed in 2017. There are new in vivo animal studies, but they do not clearly show evidence of developmental toxicity.

The discussants today have done a great job of taking us through a lot of the literature, so there is a vast voluminous literature, let's agree. There are many studies that report multiple outcomes, many implicating non-cholinergic pathways, but these are not identified by or -- identified confirmed mode of action. It would by my contention that the field of toxicology has moved beyond descriptive toxicology. We need to continually push ourselves and understand what that mode of action is.

Many of alternative non-animal approaches can be useful screening tools, but do not provide clearly shown

through scientifically valid testing evidence of effects in mammals.

As a footnote, I would let the panel know I chaired the Society of Toxicology's 2015 Future Tox 3, in which we looked at a number of new approaches so that we can continue to bring in new approaches and tools for to progress the science.

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DR. JUBERG: What do we have through with chlorpyrifos, relative to the guideline studies that were referred to by a previous speaker. There are four studies and three animal species. We're required to do two. We did an extra one in the mouse, done according to this test guideline. Collectively, what were the study conclusions?

No developmental toxicity in the absence of maternal toxicity. Cholinesterase inhibition was the most sensitive endpoint in all studies. And I point this out because it's been pointed out by a number of scientific advisory panels and others that protection against ChEI, or cholinesterase inhibition is protective against other potential toxicities.

And I would make note that in addition to some of the reviews that have been cited, we started looking at this in 2008 with the Eaton et al. publication followed up by a very robust review by an Abbi Li published in 2012,

in which she looked at a lot of this literature itself.

Another review panel was Prueitt et al. 2012. So I would hope that the panel would take a look at some of those evidences as well.

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DR. JUBERG: So when you -- we talk about, and you discussed this quite well, when we look at this -- all the new studies provided to the DARTIC, there are a number of studies, hundreds. And you can see the list in which they were provided, two revised human health assessments, including also we have September 1 and 8 list. They do number in the hundreds. But I would say that many of the studies do not meet sufficient relative to animal testing. If you parse through these, and I did, look at each study, numerous studies did not even use chlorpyrifos as the test material, many involved postnatal exposure only, a number looked at non-developmental endpoints.

So the ones I focused on, and that are in our written comments that were submitted to you, were those involving gestational exposure primarily. However, many of the studies here even, there are experimental elements that would conflict with criteria for what would be considered sufficient animal evidence.

What are some of these design challenges? I would contend that a non-relevant route of administration

for humans, subcutaneous injection is a challenge. The use of dimethyl sulfoxide, a number of -- there's great literature on the low levels, even at levels of 0.0025, in which this is neurotoxic.

The use of a single does, or high doses, which exert frank toxicity, or singular doses don't permit the evaluation of a dose response. And, in fact, if you look through the literature, there are many studies that report effects on offspring below a threshold for cholinesterase inhibition. But as I go through these, many of the studies don't ever mention or don't measure cholinesterase inhibition. So while it's assumed that 1 milligram per kilogram day is that threshold, I think we need to look at where you're inhibiting plasma, and then red blood cell, and then brain cholinesterase.

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DR. JUBERG: So this is just an example. I've made a table. I've not -- I've just pulled six of studies from this OEHHA September 1 list. They're organized chronologically by date. The rest of the studies are in our written comments. What I want to just take you through is just showing you some of the challenges in my mind of what these experimental challenges are.

If you look at the route of administration, many involve subcutaneous injection, not irrelevant. Dermal

exposure is very relevant to humans, but not subcutaneous injection, the use of a challenging vehicle, the use of either singular doses, or look at the top one, 200. That's approaching the lethal dose for chlorpyrifos.

Such that if you have a singular dose, you cannot use dose response or evaluate dose response relationships. And, in fact, many of these studies did not measure cholinesterase inhibition.

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DR. JUBERG: So I just have one slide of quotes. The 2012 Scientific Advisory Panel took a look at a lot of this literature, and they just noted the same things that I've mentioned. They had concern about the use of DMSO as a vehicle, because of its intrinsic toxicity.

They also, and I think the second quote is particularly relevant to the discussion today. They recommended that these experimental outcomes talking about a number of the studies that you've reviewed, be regarded as exploratory and hypothesis-generating, as opposed to being evidence of toxicity.

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DR. JUBERG: Let me talk about the definitive study we've done to evaluate developmental neurotoxicity that was referred to by one of the previous presenters. This is an animal study required by U.S. EPA to evaluate

the potential for neurodevelopmental -- or developmental neurotoxicity and neurobehavioral neuropathological observations in the offspring. This is a different type of study than just a straight developmental study that we've conducted in rat, rabbit, and mouse.

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DR. JUBERG: This study is specifically required to address an inappropriate animal model, the types of observations and outcomes that could be or are often reported in some of the epidemiological studies. You can see the dose ranges there, one involving a fairly low dose of 0.3. This is consecutive dosing for 24 days. The outcome, in this case, were normal learning and memory, as evaluated by those two specified types of assessment, and then habituation.

It's important in this DNT study, and for me to relay to the panel and to the audience, that we looked extensively at brain. We looked at brain weight, histopathology, and morphometrics, or measurements, on nine brain regions. And you can see those there.

The authors concluded, and this is a published study in Tox Sci that there is no evidence of selective neurodevelopmental toxicity in the absence of maternal toxicity.

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DR. JUBERG: Now, since 2008, we were required to conduct, and this is my last slide, on the testing that we've done. It's a comparative cholinesterase assay. And it specifically required to examine life stage sensitivity to cholinesterase inhibition, specifically over the lower portions of the dose response curve. So we, in fact, not only use three different vehicles, but we actually went below where EPA required, so we went down to 0.1.

Following acute and repeat dosing to both chlorpyrifos and the oxon metabolite, which has been discussed, the no observed effect levels were the same across age groups for both brain and red blood cell cholinesterase inhibition. The reason I highlight this final point then is that there's consistent evidence across different types of studies, developmental, the developmental neurotox, the comparative cholinesterase assay that demonstrates that fetuses, or the young, are less sensitive than dams or mothers. That was also a study that's been published.

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DR. JUBERG: So, in conclusion, I would state that results from scientifically valid testing, according to generally accepted principles do not indicate developmental or neurodevelopmental toxicity in the absence of maternal toxicity.

I will agree that there are many experimental studies that exist, but they do not meet the criteria for sufficient evidence in experimental animals, mammals, such that extrapolations to humans is appropriate.

In conclusion, there are no new animal data to justify listing chlorpyrifos as a developmental toxicant.

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DR. JUBERG: Collectively then for my colleagues, we would contend that none of the three criteria are met for the DARTIC to recommend listing chlorpyrifos to cause developmental toxicity. The epi studies do not provide sufficient evidence in humans that chlorpyrifos causes developmental toxicity, neither the epi studies, nor the animal studies provide limited evidence, or suggested evidence in humans that chlorpyrifos causes developmental toxicity. And finally, the animal studies, while a number of new voluminous studies exist, do not provide sufficient evidence in experimental animals -- mammals that chlorpyrifos causes developmental toxicity.

With that, I thank you, and I will stop.

CHAIRPERSON GOLD: Thank you.

Are there any questions for any of the Dow commenters by the panel?

Dr. Pessah.

COMMITTEE MEMBER PESSAH: So it's well known that

rodents have very high carboxy esterases that don't really model to the human condition. And you seem to favor that last study you presented, so I guess I have to ask the question. Did you account for underestimating potential toxicity due to the very carboxy esterases, especially at the low doses?

DR. JUBERG: In the CCA study?

COMMITTEE MEMBER PESSAH: No, in the animal studies, the Marty et al. that you referred to at the end.

DR. JUBERG: Did we look at carboxy esterases?

COMMITTEE MEMBER PESSAH: Did you take into account the fact that carboxy esterases were much more abundant during the developmental trajectory.

DR. JUBERG: No, I believe we did not.

CHAIRPERSON GOLD: Any other questions or comments from the Panel of any of these commenters?

Mr. Landfair.

MR. LANDFAIR: Dr. Gold, again we do have documents for you to supplement your thinking and consideration. I'm happy to just bring them to you at the next convenient point, but we're going to request that you not make a decision until you hear what the other agencies have said about this very question.

CHAIRPERSON GOLD: I wonder if counsel wants to advise us.

CHIEF COUNSEL MONAHAN CUMMINGS: So we had a request -- a very late request from DOW to provide information to you. And we advised them that they could bring whatever they wanted to to the meeting to provide to you. It's up to you whether or not you consider it, and to what level you consider the information you're being provided. But the same is true for the slides of the present that wasn't able to stay, you're going to have the slides from that too. And it's up to you to what extent you consider that information.

CHAIRPERSON GOLD: So I'll suggest that maybe these things get distributed to us before we take our break, which we're going to do now, because I see the energy level is kind of waning here.

(Laughter.)

CHIEF COUNSEL MONAHAN CUMMINGS: What we might -- what we might want to do is we can hand those out to you, but I do want to remind the group that when you go to lunch, that -- I mean, it's okay if you want to sit through lunch and read some stuff. But I -- you can't talk to each other, have any kind of discussion about this chemical, or the other one that you're considering today among yourselves or with others. And if somebody comes up and talks to you, you need to disclose that when you come back to the meeting today.

Thank you.

CHAIRPERSON GOLD: Okay. With that, I'm going to thank everyone for their patience, and we will take a lunch break of 45 minutes, so returning here at about 1:35.

And let me just say that we have approximately nine requests for public comments on chlorpyrifos, and we will take those up after lunch.

(Off record: 12:53 p.m.)

(Thereupon a lunch break was taken.)

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

(On record: 1:41 p.m.)

CHAIRPERSON GOLD: Okay. We'd like to reconvene, if people would please take their seats.

Okay. At this time, we're going to continue with the public comments, and we actually have 10 now. And some are -- I notice that some are from the same organization, so if you want to, in the interests of time say "me too", or "I agree" or whatever, that's fine. But if you want your five minutes, that's also fine.

So first, we have Katherine Foster, is she here?

Katherine Foster?

DR. FOSTER: Yes. Thank you so much for the process and the work you're doing. I am a pediatrician. I'm also on the Environmental Health Sector of the American Academy of Pediatrics, and the Environmental Health Committee of the Bay Area Chapter of Physicians for Social Responsibility. I've been a public health advocate my entire career. I was a Sonoma County First Five Commissioner for 11 years, and also as a medical consultant to a regional center where we take care of children and adults with developmental delays.

Developmental delay implies you're going to catch up some day, but the fact is they don't. Most of the brain damage we see is permanent and life long.

There is a very strong consensus in the medical community that chlorpyrifos is toxic, and that includes the American Academy of Pediatrics, the Child Neurological Society, Physicians for Social Responsibility, the American College of Obstetricians and Gynecologists, the Endocrine Society -- do I need to go on and on? There is a strong consensus that doctors in this country identify chlorpyrifos as toxic to the brain of not just children, but adults as well.

I wanted to quote Dr. Tracey Woodruff who's also on our Physicians for Social Responsibility who's done a study on reproductive health and environment at the UC San Francisco. And she said there was a reason they focus on pregnant women and children. Exposure to even tiny amounts of toxic substances can have outside effects. Exposure to toxics is especially detrimental to fetuses, as well as infants and children. And if you prevent the problem at the beginning, you'll get a lifetime of benefits.

Well, the converse of that is also true. If you don't intervene at the beginning, you get a lifetime of disability. The costs of that both to families and to our social institutions is more than we can measure. In dealing with autistic -- people with autism well into adulthood are unable to be productive citizens. They're

unable to be employed. It goes on and on and on.

And I do also want to address a consensus statement by health specialists who feel like the -- who felt that the evaluation that we do for toxins is not adequate. We have many findings of toxic elements in our food chain and water chain. We assert that the current system in the United States for evaluating scientific evidence in making health-based decisions about environmental chemicals is fundamentally broken.

To help reduce the unacceptable high prevalence of neurodevelopmental disorders in our children, we must eliminate or significantly reduce exposures to chemicals that contribute to these conditions.

You must adopt a new framework for assessing chemicals that have the potential to disrupt brain development and prevent the use of those that may pose a risk. This consensus statement lays the foundation for developing recommendations to monitor, assess, and reduce exposures to neurotoxic chemicals. These measures are urgently needed if we are to protect healthy brain development so that current and future generations can reach their fullest potential.

And it's signed by about 20 different medical associations.

And I can -- and I not only thank you for what

you do, I thank that electorate of the State of California who voted for Prop 65 in the first place for this exact scenario. We do not want poisons in our food and water. And I implore you to listen. And I know we live in an age that's not validating science very much. We've come on hard times. But those of us who believe the science believe that chlorpyrifos is part of the problem. And I implore you to add it to the list of toxic chemicals.

Thank you.

CHAIRPERSON GOLD: Thank you.

Are there any questions from the panel for Dr. Foster?

Our next speaker is Ann Lopez. Is she here?

Thank you. Also five minutes.

DR. LOPEZ: Yes. Hello. I'm Dr. Ann Lopez. I'm the Director for Center for Farmworker Families. And I also do studies on the environment. I'm an environmental science Ph.D. And having studied farm workers for the last 20 years, and finding out what their needs are. Almost every farmworker family I've ever discussed the issue of education with tells me that they want their kids educated, out of farm work, and having a better future. And that dream is their hope for the children's future and what gets them out of bed and in the fields from between 5:00 and 7:00 in the morning.

So in the Salinas Valley, I see that we have one of the worst examples of environmental racism in this entire country.

First of all, we, expose Latinos, most -- and the Salinas Valley is predominantly populated by Latinos. We expose them to various organophosphates. I personally have read the CHAMACOS study. I'm very impressed with it. And if what they say is true, then many of the children are born and already will experience intellectual deficiencies.

Then they go to school, and the school surrounding the fields, and when chlorpyrifos is used in the field, it -- since it's drift prone, it moves into the classroom. Teachers complain about it all the time, and effects the development of their children's brain.

So now the children are impacted on two fronts, prenatally and also in the classroom, and eventually become intellectually deficient. How are they supposed to be successful and develop a better life? I just think it's absolutely unconscionable and cruel.

And the interesting thing to me is that in the Salinas Valley, the chance of a Latino children -- the chance of the children and the students being Latino is 3.2 times higher than being white. And this is borne out in Santa Cruz County. Because in Santa Cruz County, it

was very interesting, because you don't even hear about what I've just told you in the Salinas Valley. And yet, they stopped the crab harvest two years ago, because the crabs contained the neurotoxin domoic acid. And I thought that was very interesting because northern Santa Cruz County is almost all white.

So you ignore where the Latinos live and the impact of neurotoxins on their brains, but you protect the people in north county that are white from a similar fate.

And I implore you to list this horrible chemical. No one should be exposed to it.

Thank you.

CHAIRPERSON GOLD: Thank you.

Any questions from the Panel for Dr. Lopez?

Okay. Our next speaker I believe is Raul Garcia if I'm reading it correctly. I'm not sure about the first name, but I believe it's Raul Garcia.

Five minutes. Please introduce yourself and where you're from.

MR. RAUL GARCIA: Hello everyone. Hello everyone in attendance and the Panel. My name is Raul Garcia. I come from Porterville, California. I am in the south central -- not south central -- in the Central Valley, in the County of Tulare. And the one thing I want to get clear is that I also don't have any financial interest,

you know, unlike some people here, but that's fine.

I have this weird thing called human health interest in mind. Not just for my community, but for the people around the world for which the canned -- for which the fruits and vegetables in our county go all over to.

These are issues that do not just affect the communities that I live in or the communities that other people around my area live in. They are issues that affect the entire world. Our food here in the Central Valley in the State of California goes all over the world, so which means that the effects of this pesticide are not just felt by those living in the San Joaquin Valley, or in the Salinas Valley, or anywhere else in the state, but these affects may also be felt around the world unknowingly.

So let's take that into consideration next time we decide to approve another pesticide or, you know, say that there isn't sufficient evidence, because the studies say this and the studies say that. Like, these are more than just studies. We're more than just people and numbers on a chart, and on the -- you know, and on the program and this and that.

I think these are people that are actually affected by this. A lot of people think, oh, I might have something like asthma or allergies when it's not. And it

could be something like the pesticides that are affecting them. You've got mothers and fathers and children that are exposed to these dangers through no fault of their own, all because they're trying -- all they're trying to do is make a living, and all the kids are trying to do is go to school and get a decent education, but that's beyond the point.

I, as outreach coordinator for the Coalition Advocating for Pesticide Safety, as known as CAPS, and on the behalf of CAPS would like to express my support for this pesticide known as chlorpyrifos to be recognized as a developmental and reproductive toxicant, and there's a Proposition 65 of California passed in 1986, and that was all. Thank you.

CHAIRPERSON GOLD: Thank you. Any questions for Mr. Garcia.

Thank you. Seeing none.

Sandra Garcia, I believe. Sandra Garcia?

Wait one moment. I'm sorry to interrupt. We do have a translator, so she will receive five minutes and the translator will subsequently translate into English so it's in the record, just so everyone knows what we're doing.

MS. GARCIA(through interpreter): Hi. My name is Sandra Garcia, and I've been working in ag fields in

Tulare County for over 20 years. And one of the things I wanted to share is that my mother was poisoned by this chemical. She's now died. For over 30 years, I've been asking for this chemical use to be reduced for you to change them to look for another alternative.

There are many choices out there that they could use for fumigation, instead of using these chemicals, so we please ask that you do this. Right now, my grandchildren are also being impacted. They're suffering from attention deficit, hyper activity, and they can't concentrate in school. And so we're all being affected by this.

And it's not just my grandchildren. It's also the children of the people I work with. They're suffering some of the same issues. And I've heard today that -- by some people that that's not related to the use of these chemicals to these toxicants. And so I ask why don't you come out there and actually look at the farm workers' children, why don't you do tests, analysis, go out there and see what's going on in these rural areas, instead of just focusing on the studies that you guys have done, because all of these have been done in mice. But it's not the same thing than going out there and seeing them and watching how these children get sick.

And then you take them to the doctor, and the

doctors can't figure out what's wrong with them. And they always end up telling you, well, you know, we don't know. There doesn't seem to be any cost.

So if you're doing all these studies and all these tests in mice and other animals, come out instead. Help out these children that need all your help. So we ask that you help us out, because at the end of the day, we, the field workers, are the ones that put the food on all of your tables, so we need your help.

Thank you.

CHAIRPERSON GOLD: Were there any questions for this public speaker?

Okay. Thank you.

The next speaker is Benny Corona.

Benny Corona?

MR. CORONA: Hello. My name is Benny Corona. I am from Tulare County. One of the counties that has the most usage of chlorpyrifos. I grew up as a farmworker. I come from a farmworker family. I'm going to try very hard not to let the emotions get in the way of what I'm trying to say. But I grew up in a farm worker family, and I've never needed a study to show me how harmful these pesticides, specifically chlorpyrifos, are to our community members.

Unfortunately, if you do go to Tulare County and

meet some of these farm workers, you will not find one person that's never -- that has not been exposed to this chemical firsthand. I have worked in the fields with freshly placed -- sprayed pesticides, not knowing, you know, that it's something that's not supposed to be done, and experienced firsthand that -- the uncontrollable coughing and the tearing up of the eyes, and just being unable to stay composed.

We -- I've never needed a study to show me that this is -- these chemicals are harmful to us. And I am deeply concerned about every single farmer worker that continues to this day working in those folds, like Sandra who just spoke.

I fortunately do not work in the fields anymore. I'm part of this -- you know, people talk about this dream that our parents had. And I -- I graduated from a university and everything. And today, I'm here because I want to speak on behalf of a lot of these folks that aren't even able to make trips to these kinds of hearings and speak on behalf of themselves of the studies that are being done that are trying to recognize whether or not the pesticides like these are hurting them or not.

I mean, if you can't defend yourself, there's really not -- if you can't defend yourself, there's really -- I mean, there's -- you don't have a voice. You

can't defend yourself, but that's why I'm here.

So I'm here to say that I do support chlorpyrifos being designated as a developmental toxicant. And I want to thank the Board for the -- I know it's a lot of hard work. Being a scientist, I've worked with scientists in the past, and I know's it tough. So, yeah, that's all I had to say.

CHAIRPERSON GOLD: Thank you.

Are there any questions for Mr. Corona?

Okay. Thank you.

The next speaker Katley Falconer.

MS. FALCONER: Thank you very much for being here, and for doing the science that is so significant in helping autism.

My grandson is autistic. And in the course of trying to understand first what it was, I secondly tried to figure out why. And I came to the realization after doing much of the reading of some of the studies you have discussed today that chlorpyrifos is a critical issue. And when I realized that my daughter had been pregnant in a brand new home built on property that originally was a Walnut Grove, and an existing continuation of a walnut grove nearby, less than 100 feet from her home, I realized that there was a significant value, because for the next five years my grandson played in a park area which was

directly across the street from a walnut grove, and you realize that chlorpyrifos is one of the key pesticides that are used in that process.

This is a silent, silent deadly material, and it is something that is hidden to many, many people within a community. I became very involved with chlorpyrifos, when I met some of the people you have heard speak today.

Coalition Advocating for Pesticide Safety brought me attention --

(Crying.)

MS. FALCONER: I am sorry -- to the people that have most significantly been involved in the deadly experience and in the health issues that are part of the pesticides chlorpyrifos.

When I became evident was that as part of a rural community of Visalia, California, I needed to go into that community to try and understand how I could relay the information about these pesticides that surround them. They have no idea. My daughter is a kindergarten teacher And for the first three weeks of the time that we were here, helping with my grandson - we live in Washington and we came down to help with the grandson - I realized that there is an issue in those classrooms.

She is one of four kindergarten teachers in Shannon Oaks School -- Elementary School. She, of course,

has raised a child who is now 7 and autistic. In that classroom, there is one IEP student that is autistic, but she recognized two more, both of which are of Hispanic background, both of which have -- none of them have the education that they should have gone in and had some sort of a diagnosis, so they could become part of an IEP program.

There were three other kindergarten classes, all of which have at least one autistic child, some are identified, some are not.

And in the course of going to the Visalia Unified School District meetings, I recognized that there is a problem that is created throughout all of the classes, and that is behavioral issues. And it roots down to the pesticides, the organophosphates, the other materials, maybe not just chlorpyrifos, but many of these other materials.

And the behavior is becoming very evidence. One teacher and one aid in a classroom indicated that she worked with three different kindergarten classes, all of which had at least one autistic child in that classroom, plus one was an additional disability. So it is not just within the Hispanic communities, it's within the rural, local, center parts of the cities, and it is becoming more and more prevalent.

In the year 2002, there were 1 out of 150 autistic children. That was the year when chlorpyrifos was removed from the residencies. However, right now, it has gotten down to 1 out of 45. This is the most recent in 2017.

I attended a talk TACA conference, which is Taking -- Talking About Carrying[sic] Autism. And in one of the presenters, Dr. William Shaw, from Great Plains Labs, said that there are critical issues that create autism. Yes, genetics; yes, minerals that you take; yes, the vitamins you take. But the environmental issue he said, up to 70 percent of it is these pesticides.

And I would like very much for you to consider the removal of chlorpyrifos and any other organophosphate that infects our foods that we eat when you'd have no idea it's on their. The residues you have no idea, plus the effect that it is happening in these children.

Thank you very much for all of your work, and I hope you will continue to consider to remove chlorpyrifos. Thank you.

CHAIRPERSON GOLD: Thank you.

Any questions from the panel?

Okay. Our next speaker is Emily Marquez.

Emily Marquez.

DR. MARQUEZ: I am a staff scientist at Pesticide

Action Network. I am a biologist, and I used to study comparative endocrinology and development before I got to PAN.

So the organization I work with we've done air monitoring over the past several years. And we do it with community members like the ones who just got up and spoke today. And I just wanted to call your attention to the 2016 EPA Human Health Risk Assessment. There's a couple sentences in one part of the document where they reference air monitoring data. And some of that data has been collected by my organization by community members who live on the front lines of pesticide exposure.

In several of those studies, they have found once using the new point of departure that they reference in that document based on the Columbia study that the levels of concern are exceeded for pregnant women and for young children.

And I just wanted to reiterate the point that Dr. Irva Hertz-Picciotto didn't get to make today, but she just wanted -- or I wanted to say that the vulnerability of the developing brain is key in these considerations. So as we -- the more we learn, the more we recognize that that's a really important developmental outcome to consider when we're looking at toxicants like chlorpyrifos.

And finally, the other point I'd like to make is just that the USDA and EPA documents that the previous speakers from Dow referenced don't represent any new data for consideration by the Committee.

Thank you

CHAIRPERSON GOLD: Thank you.

Any questions from the Panel?

Very good.

Our next speaker is Carole Erickson.

MS. ERICKSON: Hello. I'm Carole Erickson. I'm from Monterey County. I'm a retired public health nurse and mid-wife, so I have a particular interest in the health of women and children.

I followed the CHAMACOS study since its inception, because it was such a breath of fresh air, somebody who was looking at the total environment and not just looking at one chemical as we've been doing here today. It's so wonderful to have you here available to talk to you about this.

Monterey County is essentially ground zero for the tonnage of pesticides, including chlorpyrifos, that is dumped on our agricultural fields. The Salinas Valley in particular is heavily, heavily hit. If you look at it on the tracking map from the California Environmental Health Tracking Program, you can zero right in. It's the reddest

of the spots in California, except for Tulare and Kern counties.

That means that unlike the discussion today about one chemical in its lab situation for most it, the mice, and then in the human situation, it's not one chemical. It's that chemical plus all the others, and they change over time. One gets abandoned. Methyl bromide is finally going out the door. But, of course, more toxic things were proposed in our county. Methyl iodide was considered to be a very good replacement by the EPA -- the national EPA.

We've fought it. We were part of the group that got a resolution from our county board of supervisors to see it off. We had a big panel discussion that must have had 400 people, Mark? -- 400 people in attendance.

And, by golly, the manufacturer decided to pull methyl bro -- methyl iodide out of the country, not just out of California.

I'd like to propose that this multiplicity of exposures, plus including chlorpyrifos, including some of the things that the Dow Chemical people said -- you know, people who don't have the means to wash their shoes off before they come home. I mean, that is really environmental and racist, just -- injustice, to talk about people who are poor somehow contributing to their own

problems. They have to work in the middle of all of that.

So, of course, the mothers and fathers also -- fathers haven't been mentioned at all, but fathers are also affected by all these chemicals.

Biomonitoring has been done to some extent in our county, but not very often. It is not on the radar screen for most practicing pediatricians, simply not up there.

The county health department does have some of that done, but it's not a complete panel. It doesn't include one-eighth of the number of pesticides that are out there.

This is a problem. How do you know what's affecting people unless you can monitor it? That's the data that everybody needs, as well as you. That's what you're looking for.

Some children were given -- some teenagers were given bracelets to wear that are sensitive and can pick up any ambient pesticide. I think there were 80 of them who were chosen. It had to be girls. The boys wouldn't wear the bracelets.

(Laughter.)

MS. ERICKSON: The result was stunning to the people who ended up looking at the bracelets after a couple of weeks. There were so many exposures.

These kids are going to school, maybe not in a

school nearby, but in Salinas. If you can't know for certain that it's not a problem, then I don't think -- all right, one minute.

If you can't know for certain which one is a problem, reducing all of them is a very good idea. The UN has come out with a recent paper, which you've probably seen, which says there's the myths that the world needs more pesticides. It was never true. Some kind of protections, yes, but alternative ones.

One anecdote, a nurse in our group, the Safe Ag Safe Schools on Monterey and Santa Cruz counties is a nurse at Salinas Valley Memorial Hospital, which is under the partnership with Stanford. A Stanford pediatric oncologist came in just to do rounds with the patients who are newly diagnosed with cancer in the hospital. He said to the nurse, a member of our group, I can't understand this. Salinas is not very big, and half the kids in our pediatric unit at Stanford are from the Salinas Valley.

CHAIRPERSON GOLD: So your time is up. Can you wrap-up?

MS. ERICKSON: Thank you very much. I'd really appreciate having an agency that can listen to this and process it, rather than listen to it and immediately reject it. That's what we have met before.

Thank you

CHAIRPERSON GOLD: Thank you.

Any questions for this speaker?

Okay. Thank you.

Next we have Kathleen Kilpatrick.

Kathleen Kilpatrick. I'm sorry if I'm mispronouncing anyone's name.

MS. KILPATRICK: Hi. Kathleen Kilpatrick. Some of your faces are familiar. I'm credentialed as a nurse practitioner and a school nurse. I also have a background as a mid-wife from long ago. I also studied occupational environmental health, toxicology, exposure assessment at the University of Washington as a graduate student, and worked on a couple of exposure assessments of children who live in the orchards of eastern Washington.

I submitted comments, which you have at your disposal. I also read all the other comment letters. And I saw that the weight of commenting was definitely in favor of declaring a ban -- or not a ban, I'm sorry, declaring chlorpyrifos a developmental toxin.

I also looked closely at the themes and a lot of the themes in terms of the science in favor of that you've already talked about, and it appears that you agree. The thing that I felt like it was underplayed that the other speakers have talked about is the disproportionate effect on vulnerable groups, which is farmworker families,

residents of agricultural communities and especially pregnant women and children.

In real life conditions, those people don't have information. They don't have engineering controls. They don't have the ability to advocate for themselves and for their children. And that's why a lot of us are here, nobody is paying us to be here.

The themes of the defenders of chlorpyrifos were a lot of them were economic. Oh, it's well tested; it's don't have any neurological effects; not based on sound science that is accepted today. Does anybody see that as kind of a recurring theme in the regulatory -- anti-regulatory approach to science?

Oh, the epidemiological studies just show correlation. Dow read their own statement. It does not clearly show through scientifically valid testing, according to generally accepted principles to cause neurotoxicity. In other words, if we set the standards there's no proof.

And even the DPR felt like the conclusions that EPA made in their 2016 report were new science. The problem with risk assessment in general is where do you set the standard? What is regarded as proof?

For the anti-regulatory chemistry -- chemical industry, they have a moving target. And unfortunately,

industrial agriculture follows along or they'll say humans aren't lab rats, the petri dish isn't real exposure conditions. We need epidemiological evidence. Oh, you have epidemiological evidence. It's not good enough. It's not what we consider to be proof.

It's true, it's very hard to prove anything with epidemiologic evidence. It can show correlation a lot better than it can show causation, and you all know that.

I had not before found the Bradford Hill Criteria. But when I did, I checked them off. And every single one was met. The only one that was a little iffy was coherence, whether it agrees with the current knowledge of disease. And I was a school nurse for years, and I can tell you that there is a lot of uncertainty about the causes of learning disabilities, the causes of autism, and the causes of ADHD. There's a lot of controversy around that, but certainly there was some evidence that was highly suspicious.

But, yes, there are still some uncertainties, and that's why I disagree with Dow strongly. I think that the uncertainty is the very reason that we need to consider the Precautionary Principle.

That is the foundation of what DPR calls new science, which is that there is not a fine line between chemical science and public health. The public health

model goes back to Hippocrates, first do no harm. And the public health model is that we have an exposed population, what are the levels of prevention. So in having that exposed population, the canary is already in the coal mine, we're into harm reduction.

Dow and big ag may not consciously intend to create and maintain a permanent underclass of learning disabled workers, but actually it's in their economic advantage to do so.

Poverty is costly. Special education is costly. Prisons are really costly. We can't let that happen. The costs are too high. DPR has let us down, so we're counting on OEHHA and the DARTIC committee to see that we need incentives to move toward a new model of food production that reduces those chemical inputs, and that protects our children.

Thank you.

CHAIRPERSON GOLD: Thank you.

Any questions for this speaker?

Okay. Our next spear is Woody Rehanek. Again, I apologize if I mispronounced the name.

MR. REHANEK: Thank you for having me here. My name is Woody Rehanek. I am a -- excuse me. I'm a retired farmworker. I worked in the orchards of Okanogan County, Washington for 18 years. At that time, they

sprayed glutathione was the organophosphate of choice. I've heard that since then in 2014 glutathione has been band as being toxic in wetland environments, and not safe even for applicators with protective equipment.

In that 18 years I spent picking apples and propping and so on in glutathione, it makes me wonder about my exposures. But today, it's not about me, it's about chlorpyrifos. And I was then a special education teacher in Watsonville, California with significant chlorpyrifos use for 18 years. I just retired.

The -- many of my students, all of my students in fact, were children with learning disabilities, problems paying attention, reading difficulties, hyperactivity, autism, lower IQ, and/or struggles with self-control. Not all of the above at once, but combinations.

I was astonished to discover that when I joined Safe Ag Safe Schools in June, that chlorpyrifos, one of the most widely used chemicals in the world is linked not directly causally, but linked to learning disabilities. Is chlorpyrifos to blame for all of my students learning difficulties? Not at all. Yet, many other variables also correlate with these symptoms, but chlorpyrifos is undeniably one of them and one that could be avoided.

I just want to share with you as some of the hidden costs of chlorpyrifos -- and I submitted written

testimony under that title under my name. Because chlorpyrifos is linked to learning disabilities, there are hidden costs in its widespread use. In the report, the State of Learning Disabilities 2014, the National Center for Learning Disabilities found that 46 percent of working age adults with learning disabilities were employed, 6 percent were unemployed, and another 46 percent were not in the labor force at all.

Ninety-two percent, they report, of employed adults with learning disabilities made less than $50,000 per year, 67 percent made less than $25,000 per year. Now, it's not about -- all about money, but it is about longitudinal developmental. Developmental disorders that often happen in utero fleshed out over time -- over a lifetime -- we're talking about life times here.

The American Academy of Pediatrics published a paper in May 2010 titled ADHD in Urinary Metabolites of Organophosphate Pesticides. It concluded these findings support the hypotheses that organophosphate exposures at level common among U.S. children may contribute to ADHD prevalence.

As a special class teacher, I found that children with ADHD are particularly difficult to teach and engage, because they're not only distracted, but can be distracted and disruptive to others as well.

I think that there will come a day when -- I grew up in a time when it was considered a conflict of interest to be paid by a company to give scientific testimony. I do believe that those -- in those values. And I will say that as a society we need to use actionable data to take action to protect our most vulnerable citizens, our children and pregnant women and our farmworker population.

The big disappointment of the DPR hearing was that they considered quote bystandards -- standers and did not take into account farm workers. As a farmworker, I take umbrage with this. And I think I speak for all farm workers that it's an environmental injustice that needs to be remedied and you have an opportunity to do that.

Thank you very much.

CHAIRPERSON GOLD: Thank you.

Any questions from the Committee?

Thank you.

Our final speaker is Lucia Calderon.

MS. CALDERON: Hi. Good afternoon. My name is Lucia Calderon. I came here today from Salinas with community members from the group Safe Ag Safe Schools. And we are a community coalition from throughout Santa Cruz and Monterey counties. We have a diverse range of members, such as teachers from Amesti Elementary in Watsonville where in 2015, 300 pounds of chlorpyrifos was

applied within the square mile that Amesti sits and over 600 pounds looking at combined organophosphates, which concerns us because these U.C. Berkeley CHAMACOS science did tell us that 522 pounds of combined organophosphates applied within a kilometer of pregnant mothers homes correlated with a loss of 2.2 IQ points in her child by the age of seven.

We also have members that are in -- from Greenfield -- farmworker families from Greenfield, where some of the highest use of chlorpyrifos around the schools is found within a quarter mile of the Vista Verde Middle School and Greenfield High School.

I attend monthly meetings of a group of mothers in Greenfield who have children with a wide range of special needs such as autism, attention problems, learning disorders, and other developmental disorders ranging from mild to very severe who organize themselves, because they were struggling to find support for their children. And unfortunately they can't be here today to speak to you because they're taking care of their kids at home.

My office is in Salinas, and many of our Safe Ag Safe Schools community members are from Salinas, where the U.S. EPA air monitors measured average air concentrations of chlorpyrifos at three times the federal health risk limit, and that's low compared to other areas of

California, such Shafter and Ripon where -- Ripon where higher concentrations were found.

I also got the opportunity to meet with the young adults of the CHAMACOS COSECHA Youth Council who told me about their most recent study last summer where pesticide-sensitive bracelets registered chlorpyrifos on the arms of Latina teens, and that was just last summer.

So we really know that it's in the air. The epidemiologic -- epidemiological studies right in Salinas show that exposure is occurring. I really commend your Committee for bringing this issue to a meeting, and for listening to the public's comments. Please use your expertise to list this chemical as a developmental toxin and to do determine truly health protective limits.

Thank you.

CHAIRPERSON GOLD: Thank you. Any questions from the Committee of speaker.

It turns out we have one more speaker. I hope I get the first name correct. Avial or Ariel Garcia.

MR. ANGEL GARCIA: Angel.

CHAIRPERSON GOLD: Sorry.

MR. ANGEL GARCIA: Good afternoon. My name is Angel Garcia. I'm with the Coalition Advocating for Pesticide Safety. We're based in Tulare County, where there's big, big ag going there.

First, I'd like to start off with what CAPS is real quick. And that's -- it's a coalition made up of teachers, organizations, groups, and most importantly impacted and concerned community residents in the county.

One of the ideas that drives this coalition is this notion of the validation of the lived experience, where everyone's experience, regardless of background is taken into account when making -- when advocating on behalf of community members, especially around pesticide safety.

Today, I'm here with my -- with my friends, with my community to support the listing of chlorpyrifos as a developmental toxin, but I'm also here to commend this DARTIC committee for looking into this. We were really disappointed with DPR's recent decision to not do -- or go forth with a ban on chlorpyrifos at the State level. However, today there is an opportunity to remedy this by listing this chemical as a developmental toxin.

I work closely with different communities in the different rural unincorporated communities in Tulare County. Some are made of up mothers that have children with developmental delays, others with autism, others with ADHD. And the reality that they live every day it's something that can't be ignored and go unnoticed.

Unfortunately, just given their situation,

they're not able to come to these hearings. They're not able to come up to Sacramento, but I am. And so I'm here on behalf of them as well to just inform this Committee that there's a lot of mothers out there, especially in rural communities like Tulare County, that are looking for strong pro-health, pro-children decisions, and listing this as a -- again, as a developmental toxin is a step in -- a step forward.

So again, appreciate this, and this is an opportunity, so let's make it happen.

Thank you.

CHAIRPERSON GOLD: Thank you.

Any questions for Mr. Garcia?

Very well. That concludes the public comments then.

At this time, we open the discussion for the Committee to discuss the topic of listing chlorpyrifos. So I'll ask the Committee members if they have any further comments they wish to make at this time?

CHAIRPERSON GOLD: Dr. Pessah.

COMMITTEE MEMBER PESSAH: I want to address one point that's been brought up several times, and that's the validity of rodent studies in biomedicine and toxicology. We've heard that clearly there's a large number of studies that relied on rodent studies. We also know that the

biology of rodents is probably underestimating the potential hazard associated with chlorpyrifos, based on clearly defined scientific evidence. That is that they have very high levels or carboxy esterases. They also lack some of the mutations and polymorphisms found in humans that confer a significant amount of susceptibility.

They also typically -- these studies do not incorporate susceptibility genes that have been found in human populations to cause certain types of developmental disorders. And so as a scientist, I have two choices: Ignore all of the rodent studies that have been done to date, both on the toxicology side and also on the drug development side. In which case, I'd be scientifically irresponsible, and that's because most of the scientific literature to date that has led to fundamental differences in how we view science and biology, and one could count the number of Nobels, have depended on rodent studies as a starting point.

And obviously, rodents are not humans. They're models. But one cannot ignore 300 studies of which a good portion of them have proper controls, are litter-based design, have looked at different susceptibility windows, and ultimately have come up with the very same conclusions that when compared to their appropriate controls, there are biologically measurable outcomes.

And so I just want to point out that I think I would have to disagree with the idea that the animal models are irrelevant in this discussion.

CHAIRPERSON GOLD: Okay. I'm going to pause the Committee discussion, because we've had one additional request for public comment from Josephina Alvarado, and I want to give her the opportunity to speak.

MS. ALVARADO(through interpreter): Hi. Good afternoon. I am also someone who used to be a field worker. And now, unfortunately, I've been disabled by disease. And I just wanted to say it saddens me to see so many brethren, so many friends, children, becoming ill because of pesticides, particularly because of -- I can't even pronounce this class of pesticides, but I know it's impacting them. They can't study. They're having issues. Their parents can't go to work many times, and so they can't earn the money that they need.

You have the power. Help us. We need to be healthier, so that all of us can move forward.

Thank you.

CHAIRPERSON GOLD: Thank you.

Any questions

COMMITTEE MEMBER ALLARD: I do actually have a questions.

CHAIRPERSON GOLD: Dr. Allard, yes.

COMMITTEE MEMBER ALLARD: So my question is actually for Dr. Pessah. I did not see --

CHAIRPERSON GOLD: Let me just clarify. So no questions for the public speaker.

COMMITTEE MEMBER ALLARD: Sorry, no questions for the -- sorry.

CHAIRPERSON GOLD: Okay. So we're returning to Committee discussion.

COMMITTEE MEMBER ALLARD: Sorry.

My question is for you, Dr. Pessah. So I did not have the opportunity to actually look at the -- I believe you mentioned sudden infant death syndrome babies, as well as in utero. So for me a lot of the considerations has to be whether indeed that chemical is present in the fetal brain, right?

And so I just wanted to make sure that I understood clearly that from the studies that you've seen, there's indeed presence in utero in the fetal brain of that chemical.

COMMITTEE MEMBER PESSAH: So there are two published studies. One actually shows data, the Frontiers in Neurology paper. I couldn't find the data, but I read the text of the paper. And in both cases, it's not a cause and effect relationship. The question that they answer is can you find chlorpyrifos in fetal brain

subsequent to sudden death?

And the answer is yes, according to these two papers, both of which have been peer reviewed.

COMMITTEE MEMBER ALLARD: Okay.

CHAIRPERSON GOLD: Any other comments by the Committee or questions among the Committee?

Is the Committee ready to vote?

Yes.

So the question before the Committee is has chlorpyrifos been clearly shown through scientifically valid testing, according to generally accepted principles to cause developmental toxicity?

So can I please see all of those who vote yes.

(Hands raised.)

CHAIRPERSON GOLD: Eight.

I assume no noes -- no noes, because we had eight.

Correct.

Any abstentions?

(No hands raised.)

CHAIRPERSON GOLD: Okay. As I see it, we have eight voting yes.

(Applause.)

CHIEF COUNSEL MONAHAN CUMMINGS: Dr. Gold, maybe we could take just -- sorry, it's me.

Hello.

Over here.

(Laughter.)

CHAIRPERSON GOLD: Sorry.

CHIEF COUNSEL MONAHAN CUMMINGS: Can we just take one very short break to add another member to the Panel?

CHAIRPERSON GOLD: Of course. Yeah. So we'll take a five-minute break.

(Off record: 2:39 p.m.)

(Thereupon a recess was taken.)

(On record: 2:46 p.m.)

CHAIRPERSON GOLD: Okay. Can we please reconvene?

Okay. Can we please reconvene?

Everyone take their seats.

Can we please reconvene?

Thank you. We'd like to get started.

Okay. I want to first introduce Dr. Tracey Woodruff, who has joined us. She's from the UCSF Program on Reproductive Health and the Environment, and she's joining us for this discussion of n-hexane.

So we'll being with Dr. Sandy.

DR. SANDY: Yes. So the second and last chemical for today for listing consideration is n-hexane. N-hexane is metabolized to methyl-n-butyl ketone and

2,5-hexanedione, two chemicals that this Committee considered and listed in 2015. This afternoon, you are considering whether n-hexane should be listed as known to cause reproductive toxicity based on each of the following endpoints, developmental toxicity, male reproductive toxicity, and female reproductive toxicity.

I will now hand this over to Dr. Donald to introduce his staff making the presentation.

(Thereupon an overhead presentation was presented as follows.)

DR. DONALD: Okay. Well, to my left is Dr. Francisco Moran. He's a staff toxicologist in the Reproductive Toxicology and Epidemiology Section. And he's going to briefly review the data that we provided to you on n-hexane.

DR. MORAN: Okay.

DR. MORAN: Good afternoon.

--o0o--

DR. MORAN: I would like to start by reviewing a previous DARTIC meeting on methyl-n-butyl ketone, MnBK, and 2,5-hexanedione, 2,5-HD.

At the November -- yeah, at the November 9th, 2015 meeting the DARTIC reaffirmed the listed the listing of MnBK as a chemical known to the Stated to cause reproductive toxicity based on male reproductive toxicity,

and added developmental toxicity as an additional endpoint.

Also, at that meeting the DARTIC listed to 2,5-HD under Proposition 65 as known to the State to cause reproductive toxicity for male reproductive endpoint.

At that meeting, the DARTIC requested that the OEHHA bring n-hexane before the Committee. The request was made because n-hexane is metabolized to MnBK and 2,5-HD as already mentioned.

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DR. MORAN: N-hexane is a widely used industrial solvent, and used also in the process of extracting oils from seeds.

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DR. MORAN: There is a metabolic association among n-hexane, MnBK, and 2,5-HD. In this schematic representation, n-hexane is metabolized by hepatic oxidases to hexanol and subsequently to 2,5-hexanediol.

2-hexanol can also be oxidized to MnBK, while MnBK and these other intermediaries -- these other two intermediaries can further be oxidized to 2,5-HD, that is the predominant metabolite for both hexane and MnBK.

The type of evidence for n-hexane on DART effects are presented in this slide. In addition to these relevant studies, Attachment 1 provide -- provides the HID

MnBK and 2,5-HD, as well as the studies included in it. The data will be presented in this order: Developmental, female and male reproductive toxicity.

This is a summary of some aspects of the experimental design for developmental toxicity studies in rats:

The exposures and dose in ppm are presented for each cited study. In this set of studies, the exposure was by inhalation at different times during gestation - indicated -- defined as GD. And in two experiments, the exposure was extended to postnatal days, indicated by PND

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DR. MORAN: In the same way here is a summary of the experimental design for the developmental toxicity studies in mice. The study by Marks et al., in 1980 is the only one where the exposure was by oral route. In this study, the animals were exposed during gestational days 6 to 15 to either a single dose or three times per day of the doses indicated in this slide.

In the other studies, the exposure was by inhalation at the doses indicated in the right column. There were also two dominant lethal studies.

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DR. MORAN: This is a summary of the results from four studies in rats presenting various developmental

toxicity effects, such as decreased fetal weight and birth weight, postnatal growth rate, cerebellar development, number of pups per litter. All of this in the absence of maternal toxicity.

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DR. MORAN: Here are four -- the result of four studies with developmental effects where the animal model is indicated below the author name is a mix of rat and mice. The developmental toxicity effects are decreased fetal weight, as well as birth, placenta, and gravid uterine weight, ossification of sternebrae, frequency of resorptions per litter, frequency of live fetuses per litter, live pups per litter.

And these studies also reported some maternal effects such as: dose-dependent lethality, body weight, decreased irritability and aggressiveness at the higher dose in one study.

In addition, the two dominant lethal studies already mentioned did not report developmental effects and they were not included in this summary table.

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DR. MORAN: This is a review of the effects of n-hexane and MnBK and 2,5-HD, the three chemicals already mentioned on developmental toxicity.

Fetal and birth weight, litter size, and

postnatal growth and neurodevelopmental effects are among the three chemicals -- are present in the three chemicals.

The citation for n-hexane effects are in the footnotes.

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DR. MORAN: This is a summary of the experimental design for the female reproductive toxicity studies. There are -- these are inhalation studies, where in two of them the exposure was during gestation.

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DR. MORAN: Here are the summary of the results on female reproductive toxicity. The animal's model and dose range is under the author names. There are ovarian effects such as:

Abnormal estrous cycle and ovarian morphology and effects on steroidogenesis.

There is an increase in apoptosis, cell death -- cell death rates and proportion of artesic follicles.

There is a decrease in the proportion of secondary follicles, number of embryos, first polar body formation, and mitochondrial membrane potential.

For systemic toxicity, it was described in two studies and are indicated on the wrong -- on the right column.

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DR. MORAN: This is a review of the effects of n-hexane, MnBK, and 2,5-HD on female reproductive toxicity. There were not female effects reported for MnBK and ovarian effects for both hexane 2,5-HD are consistent among the -- between both.

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DR. MORAN: This is a summary of the experimental design for male reproductive toxicity study in rats. Only one study was by oral route (Linda et al. in '92), while in all the other studies exposure was by inhalation, at different length of times indicated in the exposure column. Exposure ranged from 1 day to several weeks and 1 study to near 14 months. The doses ranged from 100 to 5000 ppm and from 10,000 to 20,000 milligrams per kilo in the oral route study.

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DR. MORAN: Here is the summary for the results of n-hexane for male reproductive toxicity. The results range from absence of effect to testicular damage, decreased sperm count, and prostate weight, and Leydig cell hyperplasia. Systemic toxicity was reported in study as decrease body weight and atrophic hind limb muscles.

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DR. MORAN: In this slide is the summary of the results for the two dominant lethal studies, where no male

reproductive toxicity was reported for this study as well as an absence of systemic toxicity.

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DR. MORAN: Finally, this is a review of the effects of n-hexane, MnBK, and 2,5-HD for male reproductive toxicity, where testicular effects were observed in -- four all three chemicals.

That concludes my presentation.

CHAIRPERSON GOLD: Thank you. Are there any questions at this time from the Panel to staff?

Okay. In that case, I have asked for a discussion of animal studies of developmental effects, and the first discussant is Dr. Woodruff.

COMMITTEE MEMBER WOODRUFF: Thank you. Thank you for the presentation. That was very useful. I was -- looked also at the same studies that you reviewed.

I first wanted to start by discussing the summary that you put together, the summary table. And I think this is something that we have been talking about in previous meetings was the quality of the summary tables and our ability to evaluate the underlying data that comes from the study. So I appreciate the presentation you did today, which is somewhat of an improvement over the tables that were in the health -- the document that we received before the meeting.

But I wanted to make an additional point that I would highly recommend using the new software that NTP and EPA is using, the HAWC project software, because I went through the studies myself and was able to actually graph all the endpoints. And I think you will find it a very useful tool to use, because while the arrows are very useful, I don't think they can always represent the actual data itself completely, so I'm going to make this request very specific about this particular software, because I know I've made this comment in the past. And so I just wanted to bring it up.

And so it would be, I think, very illuminating for you to do these graphs, and also you can import the data to do meta-analyses now. And I was just having a -- actually on the way up here, I was -- or this morning, I was having a conversation with the developer of that software at NIHS, Andy Shapiro, because I think one of the other things that we've discussed at these meetings is the ability to take this animal data and do meta-analyses with him, because as you pointed out in - and I will discuss in my comments - all the -- almost all the studies in animals saw decrements in birth weight pretty consistently.

There was various conclusions in the papers about the quote significance of those findings. But I believe if -- several of the studies found significant

correlations between -- and in a dose response fashion. And I think if you actually had the meta-analysis capacity, that you would see an overall statistically significant relationship, if you aggregated the findings across all these studies.

I would also reference that the National Academy of Sciences just came out with a report this past summer, which used some of this type of analytic tools to also do the same type of thing. The report is evaluating low dose effects of endocrine disrupting chemicals. And that -- those tools I think would be really well integrated into what you guys are doing here, because I think it would help us be able to see the data a lot more clearly --

So that's my first comment.

So I --

CHAIRPERSON GOLD: Excuse me --

COMMITTEE MEMBER WOODRUFF: Please.

CHAIRPERSON GOLD: Dr. Donald has a comment.

COMMITTEE MEMBER WOODRUFF: Yes.

DR. DONALD: Yes. I just wanted to know that we are aware of your request that we use the HAWC software. We are looking into doing that for future hazard identification documents. In the timeframe we had for n-hexane, and given that we -- it was essentially a continuation of the consideration of methyl-n-butyl ketone

and 2,5-hexanedione, we chose to present the data in the same way as we had for those chemicals. But we are -- we are --

COMMITTEE MEMBER WOODRUFF: Oh, that's excellent.

DR. DONALD: -- as I say, for future hazard identification documents we're investigating it.

COMMITTEE MEMBER WOODRUFF: I'm sure excited because the graphs are really -- the graphing capability is very exciting for those of you who like those kinds things, like myself.

(Laughter.)

COMMITTEE MEMBER WOODRUFF: So -- but I -- yes, and I appreciate also that my first comment was that we did ask to have this chemical brought up, n-hexane brought up, because the metabolites were found to be reproductive and developmental toxicants, or reproductive toxicants in the case of 2,5-HD. So with that premise in itself, it makes a lot of sense to look at n-hexane.

I wanted to comment on -- you commented on the findings from all the studies, which was consistent with the -- my own review of the findings. I would say that the studies, unlike maybe a couple years ago where some of the studies were older, I thought the quality of the studies have -- commented on were improved compared to some of the studies that we've evaluated in the past.

And I thought that the studies were mostly of reasonable quality I will note that because they're all animal studies and experimental design, this gives them an advantage, because you have both controls and animals dosed.

And the other thing I wanted to note was that there was -- while some aspects of the studies were not always well described in terms of, for example, blinding, there were some of the studies that did mention that they had had some randomization for exposure.

Also, all the studies had -- almost all the studies had a very well described exposure -- well described the exposures that they were using, and the exposures were generally of high quality.

I also wanted to comment on the -- I agree that there was consistently found that all the studies pretty much uniformly found decrements in birth weight after maternal exposures to n-hexane. There was one study, the Mass 1988 study, which was, I think, commissioned, or an NIHS study, who they actually went into evaluate the potential for the relationship to be due to maternal effects. And they did actually look at the extra gestational weight gain, which is the body weight at the time of sacrifice minus the 0 day -- the weight gain at the -- the weight at the when they did -- the weight at

the beginning, and they subtracted the gravid uterine weight, and they essentially saw that there was no change in that.

So essentially, whatever effects were going on in terms of maternal changes and maternal weight gain were due to effects on the uterus and not on the mother.

They also noted that there was lack of treatment-related effects. It was also substantiated by the fact that the weight gain of the virgin females was not affected by exposure to n-hexane.

The other item about the summary of your studies is that most of the studies were inhalation studies, and there was -- even if there was some effects at the very highest end of the doses, most of the inhalation studies were below that 5000 ppm, where they did see some type of effects. There was consistency across the rats and the mice. So there was support -- a lot of supporting evidence for effects on birth weight.

And the other thing I wanted to say was -- the other thing I wanted to say was there were two studies, which I don't know if you mentioned this, but one study actually was -- or this Stoltenberg-Didinger study looked at prenatal exposures to n-hexane and were evaluating effects on brain development. Did you mention that in your presentation?

DR. IYER: (Nods head.)
COMMITTEE MEMBER WOODRUFF: I don't think so.
DR. MORAN: What exactly that they were prenatal and postnatal?
COMMITTEE MEMBER WOODRUFF: They did --
DR. MORAN: Is that what you're referring to for those studies?
COMMITTEE MEMBER WOODRUFF: Yeah.
DR. MORAN: Yes, I mentioned it.
COMMITTEE MEMBER WOODRUFF: Yeah. Okay. So --
DR. MORAN: Are the two highest doses, I mean, the median and the high does it was prenatal and postnatal for 20 and 30 days --
COMMITTEE MEMBER WOODRUFF: Right.
DR. MORAN: -- at 800 and 1000 ppm.
COMMITTEE MEMBER WOODRUFF: Right. And they saw the effects on -- by they -- that one was actually look -- they looked at effects on the neurodevelopment. And there were -- two of the studies you mentioned, in terms of developmental, they were -- after the prenatal exposures, they looked on the -- at the female reproductive effects on ovarian development in the offspring, and also finding effects.
Let me see if there was anything else.
You say there was some indication of resorption,

but that was not consistent across the studies nor of fetal malformations, not as consistent as there was with the low birth weight findings.

That's all I have for now.

Thank you.

CHAIRPERSON GOLD: Thank you. Any questions for Dr. Woodruff?

So our second discussant is Dr. Pessah.

COMMITTEE MEMBER PESSAH: So thank you for the summary both of you. The only study that caught my attention, and probably because it was based on possible developmental neurotoxicity was the Stoltenberg. There were two papers, one in 1990 and one in 1991. And I tried to sort of make sense of this. And it took me some time, because I'm not an anatomical pathologist.

And, yeah, so there were clear effects in the cerebellum that didn't seem to map onto maternal issues. But I think it was a rather detailed study in 19 -- which one was this? The 19 -- in neurotoxicity in 1991, where they actually showed, not only histopathological slides, but did the analysis in the paper. But I'm used to actually seeing summary data.

And all I saw there was a few sections. And so I wasn't sure about how reproducible this all was. And like I said, I can't read the slides, so I just took them at

their word.

The one thing in the 1990 paper, which was maybe a prelude to the 1991 paper, I noticed that their conclusion -- and I'm going to read it. "As for developmental of the central nervous system, it could be shown that not only prenatal exposure to n-hexane does not induce a reduction in brain weight in the offspring of exposed mothers", which I had to think about that one.

(Laughter.)

COMMITTEE MEMBER PESSAH: "As for development of the central nervous..." -- I'm sorry. And then it follows, "Considering the reduced body weight of the animals prenatally exposed to n-hexane, it becomes apparent that we are dealing here with harmonious hypotrophy". And maybe you can explain that Charlie, but I can't, which I think means it's just because they were underdeveloped you would expect those effects.

COMMITTEE MEMBER PLOPPER: Yeah.

COMMITTEE MEMBER PESSAH: That's it. Okay. So I would say that, you know, what caught my attention is that clearly these were delayed in development, but not due to maternal factors is what I'm --

CHAIRPERSON GOLD: Okay. Any comments or questions for Dr. Pessah or Dr. Woodruff?

Okay. The second topic is studies of male

reproductive effects. And our first discussant is Dr. Plopper.

COMMITTEE MEMBER PLOPPER: Well, Dr. Moran did a very nice job of summarizing the exposure conditions for the studies that are involved here, as well as the findings. And I want to point out that what we have here are two different conditions for exposure. There's one exposure that was in gavage. The others are all by inhalation. And that two of them, the inhalation studies are for only 4 days or 5 days, and the gavage is only 1 or 5 days.

And I think one of the things that's key here, and I think the study by boss that looks at what happens to hexane when it's inhaled, and where it's distributed, and how the metabolites shows it, these are key -- one of the key issues here is the time course. And when these studies are for longer periods of time, where the exposure is for an extensive period of time, all of these studies have found some sort of a damage to the -- to something in the testis.

And I think that one of the problems I had with this is that the only one of the five studies that looked at either sperm morphology or some sort of testicular or other reproductive pathology, the only one that didn't show any significant changes is the one that was for the

very short period of time.

And once you start thinking about the dynamics here, and I think it's -- Dr. Pessah already outlined this problem with the previous chemical, the same thing for hexane, it's a metabolized compound that could be metabolized in a variety of places, and it could be distributed to a variety of places.

And so when you get an exposure like this, particularly when it's an inhalation exposure, I can assure you from my own past experience the time frame before it actually becomes a toxicant to other organs within an individual is significant.

So I would say that there was concern. I had it first, because there was no -- one of these studies found there was no toxicologic response. Well, that's the one at the 5 days. We don't even know if that -- the concentrations of the metabolites that might be toxic would actually get -- be high enough to cause a change, and knowing that what you're focused on is the testis in an adult male, which is actively turning over at very high rates, a short time like that and then an assessment later, it may have already healed itself for the short time. It's the long time that could be the problem. That's my opinion.

I was also concerned about the fact that one of

these studies, the longest exposure study actually ended up with an hyperplastic response in the testis that didn't necessarily have to do with reproduction, but was even worse, because that's something that's not going to be reversible.

I would point out one of the things that I had a problem with with these studies is that the two studies that actually asked the question if you're -- if this exposure occurs, is this going to affect the ability of the exposed male to actually reproduce. It did not actually assess what the impact on the testis was of the animals that were tested for fertility. And, in fact, apparently there was no, what you could say, was there was no reproductive effect.

But if you look at the details of what was said about the composition of the damaged testis or the organization of the sperm there, there are always some viable sperm there anyway.

So that doesn't -- you know, you would -- what I would have wanted to know from either one of these other two studies, okay, so after they did the breeding and they were successfully impregnated females, what was the pathology of the testis at the time this occurred?

And, of course, that isn't in here, so that is sort of -- it makes it a little bit more difficult to

assess it, but I just would point out again that except for the two studies, that -- or three studies that used very short time frame for exposures, all the rest of them found some sort of indication that male reproduct -- the male reproductive system was toxicologically damaged and that's it.

CHAIRPERSON GOLD: Thank you.

Any questions for Dr. Plopper?

Okay. The second discussant si Dr. Auyeng-Kim.

COMMITTEE MEMBER AUYEUNG-KIM: So I pretty much came to the same conclusions as Dr. Plopper. The other thing that I noted with the studies was that all the studies that had effects were all in rats, and the studies that did not have effects were in the -- in mice.

And so I tried to see if there was like maybe a metabolism difference. But it may also be the fact that in most of the -- in 2 of the 3 mice studies that those were the short durations, and so we weren't going to see -- the exposure time period was not appropriate.

The other thing that I noted was that in the rat studies was that there was only -- there was no dose response tested. It was just a high and the controls. And so -- and that was something that I saw that was a deficient in the studies, but I do agree that, you know, there was clearly an effect on the male param -- male

parameters in the study -- in the rat studies.

CHAIRPERSON GOLD: Thank you.

Any comments or questions?

Okay. The final topic is female reproductive effects and Dr. Luderer is going to be the first discussant.

COMMITTEE MEMBER LUDERER: Dr. Luderer is jumping the gun here.

So of the papers that we had for review four of them that dealt wit the female reproductive -- that female reproductive toxicity of n-hexane were from the same research group at Fujian Medical University in China. And then I'm also -- I'll talk about those first, and then there are two papers that evaluated female reproductive toxicity of 2,5-hexanedione, which we've already heard why that is relevant to the reproductive toxicity of n-hexane.

So the -- and I should also note that there are no epidemiological human studies on the female reproductive toxicity of either chemical.

So there were two papers by Li et al. that were already mentioned under the developmental toxicity discussion, which appear to refer to the same group -- groups of pregnant Wistar rats that were exposed by inhalation, gestational days 1 to 20 to 0, 500, 2500 12,500 ppm for 4 hours a day. So one thing that I noted

was that there was a table that described the data on live pups per litter, and its numbers, and the standard errors were identical for 0, 500, 2500, and 1250[sic] ppm doses. So I assumed those were the same rats, or else that would have been extremely unlikely to have occurred.

They -- the second paper added another dose group, 100 ppm. And there was -- and the other data other than the data on live pups per litter in those two papers were different. So they examined different endpoints, other than that one endpoint.

The 2014 paper reports on ovarian follicle counts in the F1 female offspring of those rats that were exposed during pregnancy on postnatal day 56. Unfortunately, they only present the data as percentages of the total number of -- percentage of total follicles in 10 sections per ovary. They provide no indication of how the sections were chosen. And the percentages of primordial plus primary follicles are in those -- in all the groups were much lower than the percentage of secondary follicles which to me indicates that there was probably considerable overcounting of secondary follicles. And they really should have presented the actual follicle count data, but they didn't.

And the other two papers on n-hexane, the papers by Liu at al. -- or sorry in the second paper Li et al.,

they also harvested the postnatal day 56 ovaries for isolation of granulosa cells, which they cultured. And then they looked at various endpoints, global DNA methylation, using the Nimblegen Promoter plus CpG island array on immunoprecipitated DNA.

And then in the other paper, they looked at mRNA and protein expression of various steroidogenic genes, and concentrations of steroids in culture media, as well as using another method to confirm the methylation status of promoters for those genes specifically.

So in the 2014 paper, they identified many differentially methylated genes among the different inhalation exposure groups, focused -- but they focused down on genes involved in apoptosis and steroid biosynthesis. And then those steroid biosynthesis genes were the focus of the other paper.

One thing I noted about the data that were presented on those steroid biosynthesis genes is that they showed strikingly similar patterns of change for all the endpoints. Progesterone measured in the media decreased -- was increased at 100 and 500 ppm and decreased at 1250. And they saw very similar estradiol concentrations were decreased at the two higher concentrations, 22,500 and 1250 ppm.

And when they looked at the granulosa cell

protein and mRNA levels of Star, cytochrome P450 11a1, which is side-chain cleavage and cytochrome P4507a1, the 17 alpha-hydroxylase, 17,21-lyase --

THE COURT REPORTER: Slow down, please.

COMMITTEE MEMBER LUDERER: Sorry.

They saw exactly the same patterns increased at 500 ppm, decreased at 1250, which is what you would perhaps expect. However, they provided very little information about the number of replicates per group for this granulosa cells endpoints. And they're also looking at -- one of the genes that they looked at is the gene that's expressed in theca cells. And these were ostensibly granulosa cell cultures, which is the cytochrome P450 17a1. And yet, they were reported upregulation of this gene in granulosa cells.

Moreover, generally, when you're culturing granulosa cells, since we know that both theca and granulosa cells are required for estradiol synthesis in order to elicit estradiol synthesis in a granulosa cell culture, generally you provide androstenedione to the cells, so that they can convert the androgen to estradiol.

And they didn't mention anything about having added androstenedione. So all of these kind of things make me wonder about the validity of the data.

The two papers by Li et al. -- Liu et al. from

that same group are also of quite -- were of very poor quality. I thought there was no mention of randomization blinding. Low -- I had low confidence in the outcome measures, particularly again the follicle count methodology, was -- even though I read it multiple times, it really did not make sense the way they described it. The follicle counts were only reported as percentages again. They used inappropriate statistics for those percentage data.

And in the 2013 paper, they appear to have done in vitro exposures to oocyte -- of oocytes, based on the text and the figure legends, but the methods only describe in vivo exposures. And the figure labels on those figures use the doses from the in vivo exposures, so -- and this was already noted in the OEHHA documents. So I think those -- basically the data are not interpretable because of all of those inconsistencies.

So that brings me to the one paper that I thought was of relatively high quality, which is on the 2,5-hexanedione. That was the paper by Siracusa et al., and they use CD-1 mice. The mice were randomized to one and half percent hexanedione in the drinking water or vehicle. And they didn't have -- they didn't have different concentrations in the drinking water, but they did expose the mice for different lengths of time,

depending on the endpoint, 1 week, 4 weeks, and 6 weeks.

And they chose their dose to cause mild peripheral neuropathy based on motor conduction velocity distal latency time on the tail nerve, which I thought was nice. They actually reported those data.

They collected the ovaries for total DNA and protein, which were decreased with the 6-week hexanedione exposure, and the ovarian weights were not significantly decreased. And then they also did follicle counts using appropriate methods at the end of the exposure intervals.

So they additionally assessed fertility. So there was a nonsignificant decrease in the total number of follicles, both in the small primordial plus primary follicles, and in the antral follicles in both of the 4 week and 6 week exposure groups, and a statistically significant decrease in secondary follicles after the 6 week exposure, and they used a non-parametric test to make the pairwise comparisons at each time point.

One thing that they didn't notice in here, the graphing software that was just discussed would have been useful, the total follicle numbers were decreased by 7 percent after the 1-week exposure, 18 percent after the 4-week exposure, and 25 percent after the 6-week exposure.

But they didn't do any kind of a regression analysis looking at maybe the interaction between time and

the exposure, which would have been nice, because it's very suggestive of a dose response.

In the breeding assay, they had not differences among groups in terms of the number of offspring produced during the first 13 weeks. But thereafter, the mice were treated -- that had been treated for 6 weeks had a significantly more rapid age-related decline in litter size by regression analysis, which might be what you would expect if you had a depletion in the follicle pool, which would then lead to early ovarian aging.

The final study was a study just of -- from the first group again, the same group that had the first -- the papers on n-hexane. And they cultured granulosa cells with very high concentrations of 2,5-hexanedione in the millimolar range, and they observed increases in apoptosis, and decreases in cell viability. But the very high concentrations I think limit the utility of that study as well.

So, in summary, there were 4 female reproductive toxicity studies of n-hexane in two species, rate and mouse. However, I thought that those have a very high probability of bias for the reasons I outlined.

And then there's one in vivo study of 2,5-hexanedione in mouse -- mice, which I think was well done and has a low risk for bias, and which provided

evidence for cumulative dose-dependent depletion of ovarian follicles with developmental exposure, with statistically significant decreases in secondary -- sorry, that one was not -- second follicles or oral dosing, not developmental exposure.

So altogether, I think there's limited experimental database that supports that n-hexane may be a female reproductive hazard, but I don't think there's sufficient evidence to conclude that n-hexane that is presumed to be a female reproductive hazard in humans based on just the one study in one species that's good.

CHAIRPERSON GOLD: Thank you. Any questions for Dr. Luderer?

Okay. Dr. Woodruff is the secondary discussant.

COMMITTEE MEMBER WOODRUFF: Thank you. That was excellent. I did note though, I think in the Li 2015 article, and maybe in the other ones, they said they randomized the ovaries just in the toxicological letters one. Anyway.

I'm not -- that doesn't take away from the other issues that you explained, but

COMMITTEE MEMBER LUDERER: The Li papers did.

COMMITTEE MEMBER WOODRUFF: Okay. I did have a -- some additional questions because when I read the papers, I noticed there were some references to two

studies that said that they had a clinical -- the Li studies were motivated because they had some occupational exposures that lead to female reproductive problems. I know I asked you for these studies which are in Chinese, but I was wondering if you had looked at those studies at all. I know they're not in our group of studies.

DR. SANDY: Well, we -- based on the fact that they were reported only in Chinese and they we're just summarized in that paper, by Li et al., I believe it was, or Liu et al., we mentioned -- we acknowledged the fact that there were some reports in humans, but we had no -- no studies. These were case reports. We have --

COMMITTEE MEMBER WOODRUFF: Well, no. Actually, you sent me the studies. So --

DR. SANDY: And we have looked at -- we asked staff to try to find out what was in those studies. And one of them there was one line that mentioned that there -- that women had menstrual abnormalities. And the other paper that purported to have reports of reproductive effects, we could not find any report in that paper.

COMMITTEE MEMBER WOODRUFF: Okay. Thank you.

I just would comment that it's sometimes helpful though in the search, because I just picked that up, even if it's in another language. I mean, it could be that it's not a very good study. But it could be that there's

something useful, so it would be helpful to have that in the literature.

The other, also again in this Li toxicological letters, they note two other papers a Huang 2011 paper, the impact of n-hexane on the secretion of mouse estrogen and progesterone. And this Cao study experimental research and sex glands of SD rats after n-hexane inhalation, which I didn't know if they would be relevant. I just looked at their abstracts actually on the way up.

And they -- so I don't -- they seem like they might be relevant to reproductive health effects. But since they weren't included in the group of studies that we evaluated, I was wondering -- I mean I don't know that we need them to make a decision, but I would just note that there might be other studies out there that once checking in the reference list of the studies that you have, sometimes that's how I found these.

I just note that when they're abstracts they said they found that -- didn't one of the studies show a progesterone effect. This -- one of the -- this Huang study also found a relationship between n-hexane on mice and progesterone, but not estrogen.

Sorry. I didn't have time to read it, because I found them on the way up in the reference listed, so I would just comment that it would be useful to check the

references next time on the papers.

DR. DONALD: We generally do. It's quite possible that we overlooked something. And, of course, that's why we give the Committee the opportunity to request any relevant papers that we have missed that they're aware of.

CHAIRPERSON GOLD: Okay. Any questions for Dr. Woodruff?

Dr. Luderer.

Comments.

Okay. Hearing none. We'll take this opportunity to look at public comments.

I have one request. Are there any additional ones?

And the one request has also asked for additional time, and we've given 10 minutes. And that's for Jay Murray to speak on behalf of the National Oilseed Producers Association, Institute of Shortening and Edible Oils, and the Grocery Manufacturers Association. So 10 minutes.

DR. MURRAY: Thank you. I'm Dr. Jay Murray speaking on behalf of all those organizations that Dr. Gold just described. It's the National Oilseed Processors Association, the Institute of Shortening and Edible Oils, and the Grocery Manufacturers Association, which submitted

written comments to you. And thank you for reading both our written comments as well as all the other materials that you were provided.

Also, feel free to interrupt me and ask questions as we go through.

N-hexane is before you today, because no authoritative body has formally identified it as causing developmental or reproductive toxicity. And this first slide is an example. This was EPA's IRIS review of n-hexane in 2005. And EPA noted some evidence of developmental effects in the Mast studies, which was assoc -- at the high dose 5000 parts per million, which was associated with maternal toxicity.

And Dr. Woodruff, I'm may have misheard what you said, but I thought this was -- you were describing this study and said that the decrease in maternal weight was explained by the uterine content. So I just pulled the study, and I wanted to make sure I remembered it right.

And so what it said was that extra gestational maternal weight gain was reduced by 23 percent, and 45 percent at a 1000 and 5000 parts per million.

So the extra gestational maternal weight is the weight after removing the uterine contents. Okay. That's not -- that's not explained by a decrease in fetal body weight. That's a true effect on the moms.

Back to the slide. EPA also described all these -- also, evaluated all these other studies, many of these are ones that you are looking at as well, and concluded that these studies do not indicate that n-hexane exposure produces adverse reproductive and developmental effects.

So many authoritative bodies have identified n-hexane as a neurotoxicant. And if the issue before you was, is n-hexane a neurotoxicant, you'd have an easy decision to make.

But the issue for you today is reproductive and developmental toxicity. There are no epidemiologic studies of n-hexane, and -- at least none that have been identified. So it really comes down to the animal studies.

And before getting into the animal studies on n-hexane, I'm going to show you one study that was not included in the H -- in the hazard identification document.

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DR. MURRAY: This is a -- and this was a study that we submitted during the data call-in. This is a two-generational reproductive toxicity study of commercial hexane, not n-hexane, by Daughtery, which showed no evidence of reproductive or developmental toxicity.

And the study is relevant, because the commercial hexane that they used for this study contained 52 percent n-hexane. Male and female rats were exposed by inhalation up to -- at levels up to 9000 parts per million for 6 hours a day for 5 or 7 during gestation days per week over two generations.

The 9000 parts per million commercial hexane means about 4500 parts per million n-hexane. There was no evidence of any effect on fertility or reproductive capacity on the histology of the reproductive organs or any other endpoint of developmental or reproductive toxicity in this study.

So again, this is not a study of n-hexane, but it adds to the weight of evidence that n-hexane does not cause reproductive toxicity.

--o0o--

DR. MURRAY: This slide summarizes the studies of male reproductive toxicity on n-hexane. And it's well understood that n-hexane is metabolized to methyl-n-butyl ketone and to 2,5-hexanedione, or 2,5-HD for short, two metabolites that are listed as male reproductive toxicants, but -- and there -- it certainly provides a rationale for taking a look at n-hexane, but it's overly simplistic to assume that n-hexane causes male reproductive toxicity because some of its metabolites

cause male reproductive toxicity.

The potential for n-hexane to produce reproductive toxicity is likely to depend on whether the rate of exposure and internal dose of these metabolites are sufficient to cause male reproductive toxicity.

In fact, there's a clear difference between n-hexane and 2,5-HD regarding the potential to cause male reproductive toxicity.

This first study Linder and colleagues at EPA conducted a short-term screening assay for spermatotoxicity in both n-hexane and 2,5-HD were evaluated in the same assay under the same conditions. Both compounds given by gavage for a total dose of 20 grams per kilogram per day, but the results were markedly different. Exposure to 2,5-HD produced substantial spermatotoxicity after just 1 to 5 doses. Whereas, n-hexane was negative in this test.

And I appreciate Dr. Plopper's comments about the duration of exposure. But, you know, this clearly indicates that there's, at least on a short-term basis, there's a difference between those two compounds.

Four of the studies on this slide were negative for male reproductive effects, and only two showed some evidence of reproductive toxicity. DeMartino being of one of those two showed adverse effects on spermatogenesis,

and an exposure level with neuropathy and weight loss serious enough to trigger premature sacrifice of the -- of some of the animals.

And the other one is the study by Nylen reported atrophy at the seminiferous tubules at a does that caused quote, "severe atrophy of the muscles of the hind limbs and reduced body weight". So -- which sure sounds a lot like peripheral neuropathy, and at least partial paralysis of the hind limbs.

So these studies don't add up to n-hexane as clearly shown to cause male reproductive toxicity.

--o0o--

DR. MURRAY: This is female reproductive toxicity. And as Dr. Luderer mentioned, the evidence for n-hexane is limited to the four studies all done at the Fujian Health College. These studies had serious limitations. Dr. Luderer has done a fine job of describing those, so I'm going -- I'm going to breeze over this slide.

The one thing I will mention from this one is that there was some serious toxicity reported in two of the studies, the first study by Liu and the first study by Li. And then both of them did a second study. And in the second study, they used at least the same concentrations at the high dose, if not higher, but never bothered to

look at maternally systemic toxicity.

One of the slides that you saw earlier said there was no maternal toxicity observed in Li 2015. The reason is is they never looked for it.

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DR. MURRAY: So developmental toxicity. This n-hexane showed little or no evidence of developmental toxicity, except at doses that produced overt maternal toxicity and even death. For example Marks found little evidence of developmental toxicity in mice at daily gava -- at total daily gavage doses of 8 and 10 grams per kilogram per day. Grams per kilogram per day.

Okay. Massive -- these are massive dose levels that cause maternal deaths and certainly exceeded the -- any recommendation regulatory agencies make about what the top dose should be.

Many of these studies do not represent scientifically valid testing because the limitations, such as unknown composition of the test material, inadequate group size, insufficient number of doses, lack of detail in methods and results, and improper statistical analysis.

So these studies do not demonstrate that n-hexane has been clearly shown to cause developmental toxicity.

And I'm going to show you two more slides very quickly here.

--o0o--

DR. MURRAY: This one you've seen before. This was in the HID. And Dr. Moran also used a slide and showed you the metabolic pathways here. And this is was -- this was the pioneering work on metabolism that was done by Krasavage at all in 1980. But there's more to this -- there is more. It's not complete.

--o0o--

DR. MURRAY: This is a more complete picture of the metabolic pathways. And the difference here is I guess the pointer is not working, or at least I can't see it. Maybe you can. But all the pathways on the right side -- thank you very much for doing that -- all the pathways on the right side were not on the first slide, neither is the pathway down to dihydroxy-2-hexanone.

CHAIRPERSON GOLD: Excuse me, the 10 minutes are up, so can you wrap it up, please?

DR. MURRAY: One sentence. In conclusion, the overall scientific evidence does not support a conclusion that n-hexane has been clearly shown to cause reproductive of developmental toxicity. Thank you.

CHAIRPERSON GOLD: Thank you.

Are there any questions/comments from the Panel of Dr. Murray?

COMMITTEE MEMBER ALLARD: I do have a question.

CHAIRPERSON GOLD: Yes.

COMMITTEE MEMBER ALLARD: I was wondering in your commercial hexane study, did you -- were you able to determine the LD50 for that particular commercial hexane?

DR. MURRAY: Certainly not in that study they would have -- not have determined the LD50. My understanding is the LD50 is probably higher for commercial n-hexane than it is for n-hexane, but I don't remember the numbers. But in that study they did not look at LD50. That was a two-gen repro study.

CHAIRPERSON GOLD: Other questions?

Thank you.

DR. MURRAY: Thank you.

CHAIRPERSON GOLD: Dr. Sandy.

DR. SANDY: If I could just clarify that in the HID on page three, we do address that commercial hexane study. And we talk about the other compounds that were in that mixture, and point out that we are -- your question before you today is does n-hexane cause reproductive toxicity? And this was a mixture.

CHAIRPERSON GOLD: Dr. Murray, you wish to address that. You get one minute.

DR. MURRAY: I'll take half a minute. Thank you for allowing me to come back up.

It makes sense. If you were -- you know, that a

study that's a complex mixture, it has other six-carbon entities. If it had been a positive study, there's no way you could have said it's clearly shown to be n-hexane, because of the other constituents in that mixture.

On the other hand, it's a negative study. And not seeing something gives you -- has some value and some utility. Now, you can hypothesize that maybe one of those other chemicals inhibits the reproductive toxicity of n-hexane, but I think there's still value in knowing about that study.

Thank you.

CHAIRPERSON GOLD: Thank you.

Any other comments or questions. The topic of n-hexane is now open for discussion by the Panel.

Comments, anyone?

Dr. Woodruff.

COMMITTEE MEMBER WOODRUFF: Yeah. I just -- one thing I forget to mention when I was discussing the studies that I wanted to point out was that the Bus study, which is a study done by the chemical industry, did look at maternal and fetal exposures to n-hexane, and they did find that the fetus is exposed to the metabolites that were already listed by the DART Panel. I'm just looking for the table here. MnBK and 2,5-HD.

So the fetus is -- I just wanted to make sure

that we also had on the record that there is documented fetal exposures to those two chemicals.

CHAIRPERSON GOLD: Thank you.

Dr. Plopper.

COMMITTEE MEMBER PLOPPER: Yeah, I just wanted to make a comment on the fact that the Nylen study that looked at reproductive toxicity in rats also exposed these rats, some of them, to toluene or to xylene to other. And in both of these cases, there was no testicular problems with the ones that were exposed together. And I think that that's an interesting problem that needs -- that I would have -- were I doing this, I would have been exploring that further, because what it means is if you have a mixture of chemicals that are in these classes, and hexane is one of them, the others may be inhibiting whatever the impact of hexane actually is. So I think that's worth of consideration here.

CHAIRPERSON GOLD: Thank you.

Any other comments?

Are we ready to vote?

Okay. So first, we'll take up male reproductive toxicity. So the question before you is has n-hexane been clearly shown through scientifically valid testing, according to generally accepted principles to cause male reproductive toxicity?

All those voting yes, please raise your hand?
(Hands raised.)
CHAIRPERSON GOLD: I see six.
Those voting no?
(Hand raised.)
CHAIRPERSON GOLD: Two.
No abstention -- any abstentions?
(hand raised.)
CHAIRPERSON GOLD: One abstention.
Sorry. Thank you.
That's right. We've got one more person. Thank you.
Okay. Next, female reproductive toxicity.
Has n-hexane been clearly shown through scientifically valid testing, according to generally accepted principles to cause female reproductive toxicity.
All those voting yes, please raise your hand?
(No hands raised.)
CHAIRPERSON GOLD: I see none.
Voting, no?
(Hands raised.)
CHAIRPERSON GOLD: Four, five, six, seven, eight, nine. Nine.
And abstentions?
(No hands raised.)

CHAIRPERSON GOLD: None.

And then finally, has n-hexane been clearly shown through scientifically valid testing, according to generally accepted principles to cause developmental toxicity?

All those voting yes, please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Two, three.

Those voting no.

(Hands raised.)

CHAIRPERSON GOLD: Four, five.

Those abstaining.

(No hands raised.)

CHAIRPERSON GOLD: I missed something.

All right. Can we please redo.

Those voting yes, please raise your hand.

(Hands raised.)

CHAIRPERSON GOLD: Three.

Those voting no.

(Hands raised.)

CHAIRPERSON GOLD: One, two, three, four, five, six.

Okay.

Any abstentions?

(No hands raised.)

CHAIRPERSON GOLD: No. Thank you.

So does the reporter need a break.

THE COURT REPORTER: (Shakes head.)

CHAIRPERSON GOLD: Okay. All right. So the next item on the agenda is the consent item. And Ms. Monahan Cummings is going to speak to us first.

(Thereupon an overhead presentation was presented as follows.)

CHIEF COUNSEL MONAHAN CUMMINGS: All right. So we're trying to make this as painless for you as possible. This item has to do with changes to the second list that's required under Prop 65, which we call the section 2700 list of chemicals that either State or Federal agencies are required to be tested for cancer or reproductive toxicity endpoints.

So for this item, we sent the Committee a staff report ahead of the meeting, and also posted that report on our website. The report looks like this.

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CHIEF COUNSEL MONAHAN CUMMINGS: Hopefully you were able to take a look at it. There's also a copy in the back of the room for the public if they wish to view it. The items -- the item that you're voting on is our suggested amendments to that report. The item is on the agenda for your consent. And this means that you just

need to vote yes or no concerning the changes that OEHHA is recommending that we make to the Section 2700 list of chemicals that need further testing, that has been -- that's based on information that we've obtained from the Department of Pesticide Regulation and U.S. EPA.

Section 2700 list is informational and has no regulatory effect.

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CHIEF COUNSEL MONAHAN CUMMINGS: So the next four slides summarize that changes that we would like to make to the list. This first slide would be these three chemicals would be removed from the list because the testing has been fully satisfied.

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CHIEF COUNSEL MONAHAN CUMMINGS: This chemical, we would remove this particular endpoint of reproductive toxicity from the list, based on information from DPR that they have that testing now.

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CHIEF COUNSEL MONAHAN CUMMINGS: These are the chemicals that we would like to add to the list, and these are the endpoints for which there needs to be further testing as reported by DPR.

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CHIEF COUNSEL MONAHAN CUMMINGS: And lastly,

there's a fairly long list of chemicals that now have been fully tested as required by U.S. EPA. So does anybody have -- let's see, let me see this other thing.

So OEHHA staff is recommending that you vote yes, so that we can make the necessary changes to the list described in the staff report. Does anyone have questions before Dr. Gold requests a vote?

Dr. Woodruff.

COMMITTEE MEMBER WOODRUFF: So does that mean for example on this list --

CHIEF COUNSEL MONAHAN CUMMINGS: I'm sorry. I can't hear you.

COMMITTEE MEMBER WOODRUFF: Do you think -- does that mean that on this list, for example benzenesulfonyl chloride, that there would be sufficient data for us to, for example, consider it as a -- look at the reproductive and developmental toxicity? What does the data requirements mean?

CHIEF COUNSEL MONAHAN CUMMINGS: They're data requirements from either U.S. EPA or DPR. I don't know whether or not that is a reproductive endpoint or not that -- because I don't have the staff report in front of me, but from time to time, we will look at chemicals that U.S. EPA or DPR have reviewed, but we don't really use this list directly for finding chemicals that we should

consider for this Committee, is that correct?

CHAIRPERSON GOLD: Dr. Donald, do you have something to add?

DR. DONALD: No, I think you covered it. Really, all this tells us is that the required testing has been completed. It doesn't tell us anything about the data that were generated by those tests.

CHAIRPERSON GOLD: Can I ask for clarification on one thing?

Dr. Pessah, do you have something?

COMMITTEE MEMBER PESSAH: I do.

CHAIRPERSON GOLD: Why don't you go first.

COMMITTEE MEMBER PESSAH: Just a point of clarification. You have nicotine and derivatives. Would that include neonicotinoids, like imidacloprid and --

CHIEF COUNSEL MONAHAN CUMMINGS: Well, this is just information as reported by U.S. EPA, so it's however they've defined that. But I don't -- I'm not aware that they give us the list of all of the derivatives that they are identifying here. So we could follow up on that, if we --

COMMITTEE MEMBER PESSAH: But I think what you're saying is it doesn't make -- it doesn't have an impact on what the Committee ultimately could review?

CHIEF COUNSEL MONAHAN CUMMINGS: Correct.

Correct. Yeah, this list doesn't really affect you all or anyone else that I can identify. It just happens to be an artifact of the law that has required it for the last 30 years. And I'm not aware that anyone uses it. Sorry.

CHAIRPERSON GOLD: One question. So the triethylene glycol, it's being suggested to remove it because it's -- the testing is partially satisfied. Could you clarify?

CHIEF COUNSEL MONAHAN CUMMINGS: So what this means is that if you look on some of the other slides, there's a whole list of different kinds of testing that needs to be done. So you can see on this last, sodium phenate. There's all these different types of tests, one of them being repro. So if you go back to this one, wherever it is, what DPR is saying to us is that the repro test is finished. There may be other ones that need to be done, but they're saying the repro test is finished.

CHAIRPERSON GOLD: I got it. Thank you.

Any other questions from the Panel?

Are we ready to vote?

Okay. So the question is based on the recommendations in the OEHHA staff report, should the Section 27000 of Title 27 in the California Code of Regulations be amended, as indicated in Section 6 of the staff report?

So if you are voting yes, please raise your hand.
(Hands raised.)
CHAIRPERSON GOLD: I see nine.
Any noes?
(No hands raised.)
CHAIRPERSON GOLD: Any abstentions?
(No hands raised.)
CHAIRPERSON GOLD: So we have nine voting yes.
Okay. The next item is staff updates, if we're not taking a break. We're going to try and charge through, right?
CHIEF COUNSEL MONAHAN CUMMINGS: Yeah, we're probably five minutes out from being done.
CHAIRPERSON GOLD: Okay. Good.
(Thereupon an overhead presentation was presented as follows.)
MS. RAMIREZ: All right. Since your last meeting, we have added a total of four chemicals administratively for causing reproductive toxicity and five for cancer.
The first slide here shows that reproductive toxicity. Vismodegib was added for all three endpoints, developmental, female reproductive, and male reproductive toxicity via the formally required listing mechanism. Pertuzumab was added for the developmental endpoint, also

by the formally required listing mechanism. And perfulorooctanoic acid, PFOA, and perfluorooctane sulfonate, PFOS, were both added for the developmental endpoint via the authoritative bodies listing mechanism.

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MS. RAMIREZ: The next slide shows that for cancer, the following chemicals were added:

Glyphosate by the Labor Code listing mechanism; pentabromodiphenyl ether mixture, DE-71 technical grade by the authoritative bodies listing mechanism; and N,N-dimethylformamide, 2-mercaptobenzothiazole, and tetrabromobisphenol A by the Labor Code listing mechanism.

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

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

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

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

CHIEF COUNSEL MONAHAN CUMMINGS: Me again one more time.

So this is the litigation update. I'm only going to talk about litigation related to Prop 65. We do have one case in the trial court that is not a Prop 65 case.

We have two cases in the California trial courts that are pretty much just waiting for related cases to be resolved because they have to do with Public Records Act requests that are related to other actions. So there's nothing new on those.

Just in the last couple of weeks, we were served in a case in the federal trial court. It's the first time that I'm aware that the office has been sued in federal court. The case is National Association of Wheat Growers versus Dr. Zeise and Attorney General Becerra. It's a derivative case of the current State court action challenging the listing of glyphosate under Prop 65. So that case was just filed, and we haven't filed an answer.

So all the rest of our cases are on appeal. We're still waiting for a decision in the BPA listing

case, which has been -- it's probably our longest running one. It was fully briefed in the appellate court in 2016. It still hasn't been set for hearing.

We have the AC -- that's ACC I. That's the American Chemistry Council I. American Chemistry Council II is a challenge to the listing of the phthalate DINP by your sister group the CIC. That one is also ready to be heard in the court, but has been setting since 2016.

Syngenta II case has to do with the listing of the triazines. And that is in the court of appeal, and -- since 2016.

The Mateel versus OEHHA case is a challenge to the current safe harbor level for lead, which is both a carcinogen and reproductive and developmental toxicant. And that is in the court of appeal. It's only been there since mid-2017, so I expect that you'll be hearing about it for several more years to come.

And lastly -- or not lastly -- yes, lastly, the other Monsanto case I mentioned is Monsanto versus OEHHA. And that's in the Fifth District. And it was -- we thought it was fully briefed, but then we've gotten a couple of recent requests for groups to file amicus briefs, friend-of-the-court briefs, in that chamber -- California Chamber of Commerce, Washington Legal Foundation. So now we're responding to those additional

briefs.

That case the court has agreed to try and fast track it, so that it would potentially be heard early next year, since the listing will be -- or the warning requirement will come into effect in July of next year. So we'll see if that actually occurs.

Anybody have any questions on those?

Thank you.

CHAIRPERSON GOLD: Dr. Zeise is going to summarize what we've done today.

DIRECTOR ZEISE: All right. So the Committee found that chlorpyrifos has clearly been shown through scientifically valid testing, according to generally accepted principles to cause developmental toxicity by a yes vote of 8 to a no vote of 0. So that will be added to the Proposition 65 list for the -- for reproductive toxicity, for the developmental toxicity endpoint.

The Committee also voted that n-hexane has been clear shown through scientifically valid testing, according to generally accepted principles to cause male reproductive toxicity by a vote of 6 yes, 2 no, and 1 abstaining. And since there are 6 yes votes, the chem -- that are required, the chemical will be added to the Proposition 65 list for the male endpoint.

The Committee voted unanimously not to list

n-hexane for female reproductive toxicity, and voted 3 yes votes to 6 no votes regarding the listing of developmental toxicity for n-hexane. So neither of those two endpoints will be reflected on the Proposition 65 list. So again, n-hexane will be listed as known to cause reproductive toxicity for the male reproductive toxicity endpoint.

Then with respect to the Section 2700, the Committee voted on consent unanimously to make the changes indicated in the staff report.

And so I believe that is it.

And I just want to conclude with some thank you's to the Committee for coming, taking time out of your very busy schedule, and all of the extensive work that was done preparing for this meeting. We really appreciate it. We really appreciate your contributions to the State.

I'd also like to thank the members of the public who attended on the web and in the room and participated in the meeting. Very grateful for all the participation. And also, I'd like to thank our staff, the OEHHA staff, both the scientific staff for the -- all of the work done preparing the hazard identification materials, our legal staff preparing us for the meeting, other staff, and our Executive Office and our Proposition 65 implementation staff for all the work they did preparing for this meeting.

So thank you all very much.

CHAIRPERSON GOLD: Thank you.

I want to add my thanks as well for the public participation and their planning and preparation ahead of time, for the staff for all their hard work in getting us ready and providing materials and being very diligent about their work, and finally, for the Panel for their obvious dedication to really doing an extremely detailed and hard work on a voluminous amount of material. So I want to add my thanks.

And with that, I think we can be adjourned today.

(Thereupon the Developmental and Reproductive Toxicant Identification Committee adjourned at 4:04 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, Developmental and Reproductive Toxicant 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 meeting nor in any way interested in the outcome of said meeting.

IN WITNESS WHEREOF, I have hereunto set my hand this 10th day of December, 2017.

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